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THE THYROID HORMONES

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with a chapter on

DISEASES OF THE THYROID

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To

Sir Charles Harington

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INTRODUCTION

THE historical development of our knowledge of the thyroid gland has already been admirably described by Sir Charles Harington in his monograph *The Thyroid Gland, its Chemistry and Physiology*[691], in his book particular attention was paid to the authenticity of the early records and for this reason the survey was confined to the works of European physicians and other scientists Spear and McGavack[1508] have also surveyed the early history of goitre, particularly from a medical standpoint Sir Humphrey Rolleston[1375] has quoted reports on the treatment of goitre many centuries ago by the Chinese but we have failed to obtain any evidence which would establish the validity of such reports We feel that an abridged version of this fascinating story would not benefit the reader and we propose to lay particular emphasis on the developments that have taken place since these books were written It is however impossible to describe these developments without brief reference to some of the historical landmarks

In 1813 Courtois[324] discovered the element iodine formed in the manufacture of saltpetre by the decomposition of calcium nitrate with burnt seaweed Seven years later Coindet[326] believing that the time honoured remedy for goitre burnt sponge might also contain iodine was spurred to treat goitrous patients with potassium iodide or tincture of iodine and was rewarded with some immediate successes At the same time Fyfe[552] demonstrated the presence of iodine in the common sponge This led to a belief now almost universally held, that goitre might be associated with iodine deficiency and in the middle of last century Chatin (see [691]) began an extensive investigation into the iodine content of air water soil and dairy products especially in the regions of France Switzerland and Italy associated with endemic goitre Chatin s analytical procedures were unfortunately inadequate for the task and led to discrepancies as a result of which the work was largely discredited However the studies of Marine and colleagues (see [691]) early this century re established the connection between iodine deficiency and goitre

During the last 30 years of the nineteenth century the association of the thyroid gland with various disorders was gradually recognized Fagge[483] in 1871 was probably the first to relate sporadic cretinism in England with atrophy of the thyroid Three years later Gull[653] described a condition in middle aged women in which many of the symptoms of cretinism were present Ord[1176] described two cases of Gull s disease which he named myxoedema and showed at autopsy that the thyroid gland of

one of them had undergone marked atrophic changes Fagge's and Ord's observations, though not conclusive indicated an association of a wasted thyroid gland with cretinism and myxoedema In 1883, Semon[1446] in a discussion of a case of myxoedema first suggested that cretinism and myxoedema have a common cause A few years later, Kocher[870] and the brothers Reverdin[1 98] published their results of a series of thyroidectomies on goitrous patients in both publications the immediate effects of surgery were reported to be good, but later the patients developed conditions resembling cretinism[870] or myxoedema[1298]

The results of thyroidectomy in experimental animals were at first complicated by the effect of simultaneous removal of the parathyroid glands [1420 546 759 462] but Gley[586] and Hofmeister[747] were later able to demonstrate the separate effects of thyroidectomy and parathyroidectomy

The suggestion made by Horsley[760] in 1890 that thyroid grafting might alleviate the symptoms of myxoedema was quickly examined by Bettencourt and Serrano[163] a lobe of sheep's thyroid was grafted in a woman with myxoedema and resulted in rapid improvement in the patient, in 1891 Murray[1131] showed that an injection of thyroid extract also brought immediate relief to a myxoedematous woman and later Fox[524] and Mackenzie[1002] found that oral administration of thyroid was equally effective Replacement therapy in thyroid deficiency in adults has since then been widely used and probably represents 'as perfect a form of therapy as any known to medicine [1064]

The beneficial effects to goitrous patients of burnt sponge and of iodine on the one hand and of thyroid administration on the other led Kocher in 1895 (see [691]) to suggest that the thyroid gland might itself contain iodine This was dramatically confirmed by Baumann[131] who found that a purified product obtained by acid hydrolysis of thyroid contained no less than 2% of iodine

The extensive work of Oswald (see Chapter 2) and that of Hutchison[780 781] on the iodine containing protein of the thyroid thyroglobulin led eventually to the isolation of the active principle thyroxine by Kendall[843] in 1915 The determination of the structure of thyroxine by Harington [689 694] finally elucidated the nature of the principal thyroid hormone The discovery of diiodotyrosine in the thyroid, which was foretold by Harington, was later demonstrated by him and his colleagues (see [691]) and Harington's early surmise that thyroxine was formed in the thyroid from diiodotyrosine has since received experimental support

Since these major discoveries two techniques have contributed enormously to our knowledge of the thyroid gland namely chromatographic analysis and the use of the radioactive isotopes of iodine of which the most commonly used now is ^{131}I The combination of labelling of iodinated compounds in the body and their chromatographic separation has revealed

many compounds which are present in amounts too small to detect by less sensitive methods By this means, monoiodotyrosine and triiodothyronine (see Chapter 2) have been found in the thyroid gland, triiodothyronine has also been detected in the plasma of humans and other animals this compound has moreover been shown to possess a high physiological potency and has come to be regarded as one of the thyroid hormones These techniques have also enabled workers to study the distribution and metabolic fate of thyroid hormones The clinical uses of the isotopes of iodine dating from the work of Hamilton and Soley[671, 672] have also been extensively studied both in the elucidation of thyroid dysfunction and in thyroid ablation in cases of thyrotoxicosis and thyroid carcinoma this work has been reviewed by Means[1064] Riggs[1305] Werner[1706], Chapman and Maloof [279] Rall [1260] and others In the present work only physiological and biochemical studies will be considered in any detail

Other branches of thyroid physiology and pathology which have advanced rapidly in the past few years relate to the effects of certain foodstuffs and drugs on thyroid function It has been known for a long time that certain foods especially vegetables of the genus *Brassica* will produce goitre in man and experimental animals Concurrently with these studies has been the discovery of antithyroid drugs which fall into two categories those which block the uptake of iodide by the thyroid gland such as thiocyanate[100] and perchlorate[1757], and those which inhibit organic binding of iodide after it has entered the gland these include the sulphonamides and derivatives of thiourea[1000, 75] The antithyroid drugs have had a far reaching importance for two reasons they have been extensively used in the treatment of hyperthyroidism[1669] and they have by their intrinsic properties enabled workers in the thyroid field to study independently different stages of thyroid hormone biosynthesis For this last reason they will be described in some detail later on

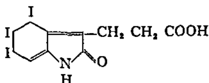
In spite of the enormous amount of work that has been done in connection with the thyroid gland and its active principle, several questions remain unanswered We do not know by what mechanism the thyroid concentrates iodine from the blood we know practically nothing about the enzymes involved in thyroid hormone biosynthesis the physical chemistry of the thyroid hormones has received little attention lastly although the overall effects of the thyroid hormones on the whole animal are well recognized the mode of action of these hormones has still to be discovered We have attempted in the following chapters to unfold the story of the thyroid hormones and to discover which hypotheses concerning their biosynthesis mode of action and the aetiology of thyroid disorders have received experimental support We have also tried to provide the research workers in the fields of thyroid physiology and biochemistry with as many references to the recent literature as possible

CHAPTER 1

CHEMISTRY OF THYROXINE

Isolation of Thyroxine (Kendall)

On Christmas Day 1914 Kendall first isolated the active iodine containing principle of the thyroid gland and reported this work briefly in 1915 (Kendall[843]) he did not however describe his experiments in detail until five years later (Kendall[844]) Kendall s original hydrolytic procedure consisted in boiling desiccated thyroid with alcoholic alkali later he substituted fresh thyroid glands for the dried material and performed the hydro lysis with aqueous sodium hydroxide Acidification gave an insoluble product which, after various purifications, was obtained as a white crystal line powder containing 65% iodine From other analytical data Kendall and Osterberg[846] ascribed to the compound the empirical formula $C_{11}H_{10}O_3NI_3$ The compound gave the pine splinter test characteristic of indoles and a deep purple red colour on treating with nitrous acid in alcoholic hydrochloric acid followed by treatment with ammonia The positive pine splinter test suggested to Kendall that the compound was an indole derivative and he named it thyroxin (In England a terminal e has been added to conform with the convention for naming basic substances) From these data, Kendall proposed that thyroxine was a trihydro triiodooxindole propionic acid with the structural formula

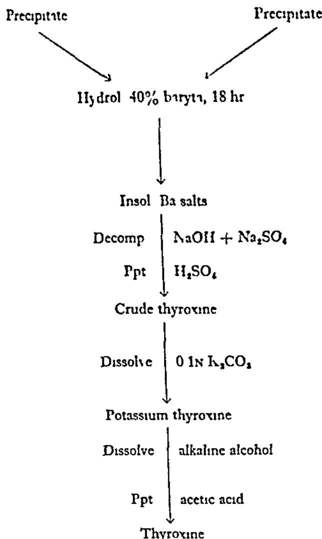


In the light of Harington s subsequent work Kendall s isolation must have involved great losses he ultimately obtained 33 g of pure thyroxine from nearly 3 tons of pig thyroid

Improved Method for the Isolation of Thyroxine (Harington)

Although Oswald[1181] [1182] had failed to obtain either diiodotyrosine or any active iodine containing compound from thyroglobulin using strong baryta hydrolysis nevertheless his success in isolating diiodotyrosine from other natural and artificial iodoproteins (Oswald[1183–6]) led Harington[683] to





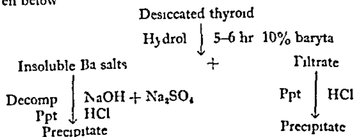
Constitution of Desiodothyroxine

Harington's[689] attack on the problem of the constitution of thyroxine was first directed towards the compound desiodothyroxine obtained by catalytic hydrogenation of thyroxine this had the empirical formula $C_{15}H_{15}O_4N$ Potash fusion of this compound at 250 C and 320 C led to different products At the lower temperature the main product was a monohydric phenol $C_{13}H_{12}O_2$ this was accompanied by some *p* hydroxy benzoic acid At the higher temperature potash fusion yielded *p* hydroxy-benzoic acid quinol and some ammonia and oxalic acid

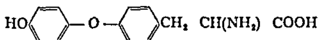
re examine baryta as a possible hydrolytic agent for thyroid substance, his preliminary experiments showed that an initial short hydrolysis with dilute baryta led to a considerable concentration of acid insoluble iodine, furthermore the material had a light colour in contrast with the dark products obtained after hydrolysis with sodium hydroxide The whole procedure worked out by Harington was as follows Desiccated thyroid was boiled under reflux for 5–6 hr with ten parts of 10% crystalline baryta ($Ba(OH)_2$ $8H_2O$) After standing for some hours the solution deposited a heavy precipitate which was separated by filtration and set on one side for further working up The filtrate was acidified with hydrochloric acid to pH 3–4 which caused precipitation of material containing 25–30% of the total iodine in the desiccated thyroid This precipitate was then subjected to further hydrolysis by heating on a steam bath for 18 hr with 40% baryta The insoluble barium salt obtained on cooling was decomposed with hot sodium hydroxide containing sodium sulphate Acidification of the filtrate from the barium sulphate with sulphuric acid gave a heavy granular precipitate of thyroxine This was purified by dissolving in seventy five parts boiling 0 1 N sodium or potassium carbonate from which the thyroxine salt separated as a white crystalline powder This was finally crystallized by dissolving in 80% alcohol containing a little sodium hydroxide and acidifying the hot solution with acetic acid

The insoluble barium salts collected from several runs of the mild baryta hydrolysis were then worked up they were decomposed with sodium hydroxide solution containing sodium sulphate and the filtrate from the barium sulphate was acidified with hydrochloric acid The precipitate obtained was then subjected to hydrolysis with 40% baryta and the subsequent working up for thyroxine was the same as that described above The purified thyroxine melted with decomposition at 231–233°C It had C, 24 14 H 1 74 N 1 87 and I 65 3% $C_{15}H_{11}O_4NI_4$ requires C 23 18 H 1 42 N 1 80 I 65 3% As a consequence of the alkaline hydrolysis the thyroxine was optically inactive The overall yield of thyroxine was about 0 12% of the desiccated gland or twenty five times that obtained by Kendall

A diagram of Harington s isolation of thyroxine from desiccated thyroid is given below



From these three degradations it was therefore concluded that desiodo thyroxine is a diphenyl ether with a propionic acid side-chain with the constitution



This was confirmed by the following synthesis

SYNTHESIS OF DESIODOTHYROXINE (HARINGTON[489])

p-Bromoanisole was condensed with phenol in the presence of potassium hydroxide and copper to give 4 methoxyphenoxybenzene this was converted to 4 (4 -methoxyphenoxy)benzaldehyde by the Gattermann method

CH_3O—Br + HO— (KOH, Cu)

↓

CH_3O—O—

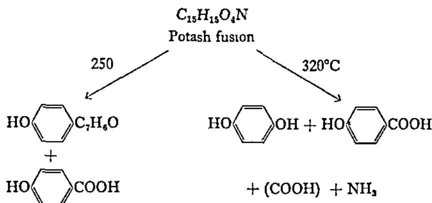
↓ KCN

CH_3O—O—CHO

CH_3O—O—CH C—CO (NH NH, CO) CH_3O—O—CH=C (NH CO, CO NH) C=CH—O—OCH_3

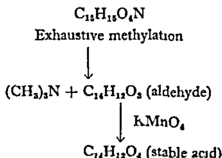
HI+P ↓ HI+P ↓

HO—O—CH_2 $CH(NH_2)$ COOH

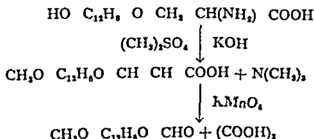


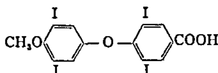
The fact that only one oxygen atom of the product of the milder fusion was phenolic together with the formation of quinol under the more drastic conditions suggested that the second oxygen atom probably united two benzene rings HO C_6H_4 O $C_6H_4CH_3$ The formation of oxalic acid and ammonia further suggested the possibility of an amino acid side chain *attached to one of the rings*

Exhaustive methylation of desiodothyroxine in alkaline solution yielded an unsaturated acid $C_{16}H_{14}O_4$ and trimethylamine, alkaline permanganate oxidation of the unsaturated acid gave an aldehyde, $C_{14}H_{12}O_3$ and oxalic acid



If the trimethylamine had come from the amino group of an α amino acid residue in the molecule then the reaction could be formulated thus





Synthesis of Thyroxine

3 5 Diiodo 4-(4'-methoxyphenoxy)benzonitrile was converted to the aldehyde by a modification of Stephen s method The aldehyde was then converted to an azlactone by condensation with hippuric acid Hydrolysis of the azlactone yielded α benzamido 3 5 diiodo-4 (4 methoxyphenoxy)-cinnamic acid, this was reduced and demethylated with hydriodic acid and acetic acid to α amino β [3 5 diiodo-4 (4 hydroxyphenoxy)phenyl] propionic acid which on iodination in ammonia gave α amino β [3 5 diiodo 4 (3 5 diiodo 4'-hydroxyphenoxy)phenyl] propionic acid (thyroxine) This compound was optically inactive as was that obtained from desiccated thyroid by baryta hydrolysis

CH_3O–C₆H₄–O–C₆H₂I₂–CN

↓

CH_3O–C₆H₄–O–C₆H₂I₂–CHO

↓

CH_3O–C₆H₄–O–C₆H₂I₂–CH C—CO (N, O, C C_6H_5)

↓

HO–C₆H₄–O–C₆H₂I₂–CH_2 CH(NH_2) COOH

↓

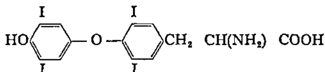
HO–C₆H₂I₂–O–C₆H₂I₂–CH_2 CH(NH_2) COOH

Two reactions were then used for the synthesis of desiodothyroxine (I) condensation of the aldehyde with glycine anhydride, (II) condensation of the aldehyde with hydantoin Both condensation products on boiling with hydriodic acid and red phosphorus gave desiodothyroxine which was identical with the product obtained by catalytic reduction of thyroxine Harington named the compound thyronine

A new synthesis of thyronine in which greatly improved yields of 4 (4 methoxyphenoxy)benzaldehyde were obtained was later described by Harington and Pitt-Rivers[696]

CONSTITUTION OF THYROXINE (HARINGTON AND BARGER[694])

The synthesis of thyronine established the structure of thyroxine except for the orientation of the iodine atoms The fact that thyroxine and diiodotyrosine both give a purple colour with nitrous acid and ammonia a property of *ortho* diiodophenols together with the likelihood suggested by Harington[689] that thyroxine was formed in the thyroid from two molecules of diiodotyrosine indicated that thyroxine would have the following formula

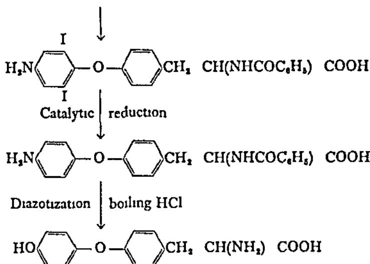


This was proved to be correct by degradation and synthesis

Degradation

(1) Potash fusion of thyroxine led to products which could not be purified The mixtures turned black on treating with alkali indicating the formation of pyrogallol like compounds

(2) Exhaustive methylation of thyroxine caused a breakdown similar to that obtained with thyronine, giving an unsaturated acid $C_{16}H_{10}O_4I_4$ With alkaline permanganate this gave an aldehyde $C_{14}H_8O_3I_4$ which could be further oxidized with permanganate to a stable acid $C_{14}H_8O_4I_4$ The constitution of this stable acid was proved by the following synthesis Triiodonitrobenzene was condensed with quinol monomethyl ether to give 3 5 diiodo-4 (4 methoxyphenoxy)nitrobenzene Reduction of the nitro compound to the amino compound followed by diazotization and treatment with cuprous cyanide gave the nitrile Simultaneous demethylation and hydrolysis with hydriodic and acetic acids gave 3 5 diiodo-4 (4 hydroxyphenoxy)benzoic acid Finally iodination in ammoniacal solution gave 3 5 diiodo 4 (3 5-diiodo-4-hydroxyphenoxy)benzoic acid the methyl ether of which was identical with the acid $C_{14}H_8O_4I_4$ obtained by degradation of thyroxine



Since thyronine obtained by this synthesis and by catalytic deiodination of L thyroxine had the same optical rotation the configurative relationship between L thyroxine and L tyrosine was proved

L Thyroxine from Aerobic Incubation of L-Diiodotyrosine Derivatives

Aerobic incubation of L-diiodotyrosine derivatives in mildly alkaline solution leads to the formation of L thyroxine derivatives this reaction will be discussed fully elsewhere Pitt Rivers[1220] showed that *N* acetyl L thyroxine obtained in this manner gave rise on acid hydrolysis to L thyroxine with an optical rotation considerably higher than that obtained by resolution of the DL compound, her sample had $[\alpha]_D^{21}$ −5 40° in alkaline alcoholic solution

Synthesis of L Thyroxine and Allied Compounds from L Tyrosine

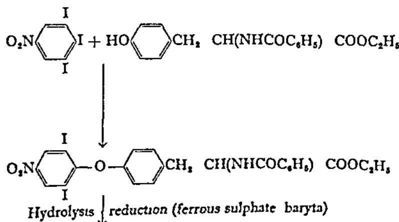
A series of researches by Hems and his colleagues a few years ago led to an excellent synthesis of L thyroxine from L tyrosine The synthesis under the best conditions worked out involved protection of the amino group of the tyrosine by acetylation and of the carboxyl group by esterification and gave an overall yield of L thyroxine of 26%[269] The reactions were as follows L tyrosine was nitrated with a mixture of nitric and sulphuric acids to give 3 5 dinitrotyrosine, this was acetylated and esterified and treated with *p* toluenesulphonyl chloride in pyridine giving 3 5 dinitro-4 toluene *p* sulphoxy-*N* acetyl L tyrosine ethyl ester Condensation with *p* methoxyphenol gave 3 5 dinitro 4 methoxyphenoxy *N*-acetyl L phenylalanine ethyl ester The nitro groups were then catalytically reduced to amino groups the product was diazotized in sulphuric and

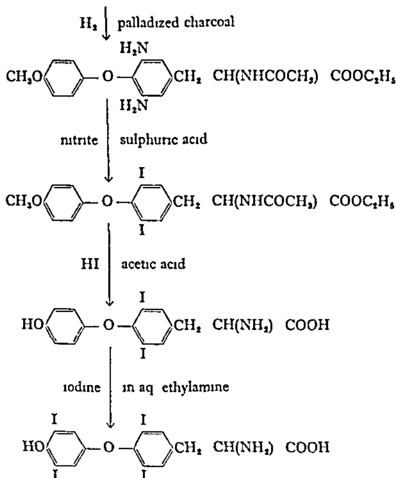
L-THYROXINE

L-Thyroxine was obtained for the first time by resolution of the (−) α-phenylethylamine salt of formyl DL-diiodothyronine[890] Hydrolysis of formyl L diiodotyrosine followed by iodination gave L thyroxine, when dissolved in 66% alcohol containing 0 16N-NaOH it had $[\alpha]^{21}_{5461}$ −3 2° D-Thyroxine was similarly obtained and had $[\alpha]^{21}_{5461}$ + 2 97°

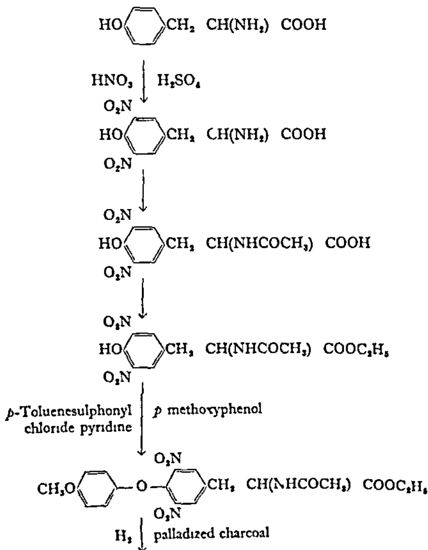
L-Thyroxine was also obtained from thyroid material by Harington and Salter[701] by successive peptic and tryptic hydrolyses, their material, in alkaline alcoholic solution had $[\alpha]_{5461}$ −3 5 Later, Foster Palmer and Leland[520] obtained L thyroxine by hydrolysing pig thyroids with pepsin followed by a glycerol extract of pig intestinal mucosa, the product thus obtained was subjected to a final hydrolysis with 2N sulphuric acid and yielded L-thyroxine with $[\alpha]_D$ −4 4° when dissolved in alkaline alcohol

The configurative relationship between thyroxine and L tyrosine was demonstrated in 1934 by Canzanelli, Harington and Randall[256] Since diiodotyrosine could not be used for the Ullmann diphenyl ether condensation, the problem was approached indirectly The first stage involved the formation of L thyronine from L thyroxine by catalytic deiodination and gave L-thyronine with $[\alpha]_{5461}$ +12 2° The second stage involved the synthesis of thyronine from L tyrosine, this was achieved as follows *N* benzoyl-L tyrosine ethyl ester was condensed with 3 4 5 triiodonitrobenzene to give ethyl α benzamido β [4 (2 6 diiodo 4 nitrophenoxy) phenyl]propionate Hydrolysis of the ester followed by reduction of the nitro group gave α-benzamido-β [4 (2 6 diiodo-4 aminophenoxy) phenyl]propionic acid The two iodine atoms were removed by catalytic reduction and the amino compound was diazotized and boiled with hydrochloric acid This led to thyronine which had $[\alpha]_{5461}$ +13 3 when dissolved in a mixture of alcohol and N HCl




H_2
palladized charcoal
H_2N
CH_3O
O
CH_2 CH(NHCOCH$_3$) COOC$_2$H$_5$
H_2N
nitrite
sulphuric acid
I
CH_3O
O
CH_2 CH(NHCOCH$_3$) COOC$_2$H$_5$
I
HI
acetic acid
I
HO
O
CH_2 CH(NH$_2$) COOH
I
iodine
in aq ethylamine
I
I
HO
O
CH_2 CH(NH$_2$) COOH
I
I

acetic acids and treated with sodium iodide, whence 3 5 diiodo-4 *p* methoxy-*N*-acetyl L phenylalanine ethyl ester was obtained This compound, on boiling for 4 hr with hydriodic and acetic acids, gave 3 5 diiodo L-thyronine, iodination of the latter was performed in 33% aqueous ethylamine and the L-thyroxine thus obtained had $[\alpha]^{20}_{D}$ −5 7 in alkaline alcoholic solution A similar synthesis was achieved by protecting the amino and carboxyl groups of tyrosine by formation of a hydantoin ring This work has led to the preparation of many derivatives and analogues of thyroxine[287, 286, 304, 426, 121, 120]



from the initial value of 8 3–8 6, when this occurs the deiodinase of the thyroid will then exert its effect We have found in this laboratory that the amounts of iodotyrosines obtained by tryptic hydrolysis of rat thyroid tend to fall, and the iodide tends to rise if sufficient buffer is not used *to maintain the pH above 8 The danger of deiodinase action taking* place is enhanced if hydrolysis is carried out in a buffer containing a volatile base such as ammonium chloride ammonia

A consideration of the distribution of iodine in the thyroid gland was made by Harington[691, 692] after the successful isolation of diiodotyrosine He concluded, after taking into account the amounts of thyroxine and diiodotyrosine obtained and the amount of iodine formed by destruction during hydrolysis that these two amino acids were the only iodine-containing compounds in the gland For some years there was no reason to doubt the validity of this deduction

MONOIODOTYROSINE

The discovery of chromatographic analysis and the availability of the radioactive isotope of iodine, ^{131}I have revolutionized thyroid biochemistry in recent years The first demonstration that these techniques might be used together for the study of iodine metabolism in the thyroid was made by Fink *et al* [481], rats were injected with ^{131}I iodide and killed 1–2 days later their thyroids were removed and hydrolysed with baryta the hydrolysates were then chromatographed in various solvents principally collidine and phenol together with non radioactive thyroxine, diiodotyrosine and iodide The radioactivity on the chromatograms was revealed by exposure on X ray film Ninhydrin was used to develop the amino acid carriers iodide was developed by spraying with silver nitrate which gave a dark spot *on exposure to light Radioactivity corresponding to the three* carriers was found there were besides other radioactive spots on the chromatograms but these were not identified

In the following year, Fink and Fink[482] demonstrated by similar methods the *presence of 3 monoiodotyrosine in rat thyroid hydrolysates this was* confirmed by Tishkoff *et al* [1810] Since then many workers have shown that after the administration of ^{131}I iodide the thyroid glands of humans and laboratory animals contain labelled monoiodotyrosine but so far no *one has yet obtained crystalline monoiodotyrosine from thyroid* substance Ludwig and von Mutzenbecher[991] obtained an iodine containing compound from the mother liquors of baryta hydrolyses of iodinated casein after removal of thyroxine and diiodotyrosine they characterized it as *monoiodotyrosine* by elementary analysis this was supported by its iodination to diiodotyrosine and its catalytic hydrogenation to tyrosine Herriott[728] has reported monoiodotyrosine as the chief product isolated after baryta hydrolysis of iodinated pepsin

CHAPTER 2

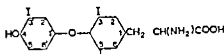
THE IODINATED COMPOUNDS OF THE THYROID GLAND

DIIODOTYROSINE

IT was not until 14 years after the discovery of thyroxine that Harington and Randall[699] demonstrated the presence of 3 5 diiodotyrosine in thyroid substance Their method entailed a step wise baryta hydrolysis followed by a long fractionation into acid soluble and acid insoluble compounds During the procedure iodide was formed, indicating considerable destruction of the diiodotyrosine The yields of thyroxine and diiodotyrosine from 250 g desiccated thyroid were 300 mg and 225 mg respectively, 184 mg iodide (as AgI) were also obtained As a result of alkaline hydrolysis the diiodotyrosine was optically inactive Later Harington and Randall[700] isolated L diiodotyrosine from a crude preparation of thyroglobulin by peptic and tryptic digestion, it had $[\alpha]_D + 2\ 75°$ in N HCl solution

Many years before Oswald[1181] had tried to isolate diiodotyrosine from thyroglobulin using concentrated baryta as the hydrolytic agent but had failed he had also attempted to extract the active principle of the thyroid by tryptic digestion Neither the active principle nor diiodotyrosine was obtained by this means These failures merit some comment The destruction of diiodotyrosine by baryta observed by Harington cannot have been responsible for the absence of diiodotyrosine in Oswald s hydrolysate Indeed Foster[519] obtained a high yield of diiodotyrosine from thyroglobulin using concentrated baryta It is more likely that the precipitant used by Oswald namely phosphotungstic acid did not bring down the diiodotyrosine from his dilute hydrolysate Foster used phosphotungstic acid to remove *impurities* from his solution which was three times as concentrated as Oswald s and remarked that only a little of his acid soluble iodine was precipitated at this stage he finally isolated diiodotyrosine as the lead salt With trypsin Oswald s hydrolyses were accompanied by the formation of excessive amounts of iodide a clue to this may be found in the discovery by Roche *et al*[1312] that the thyroid possesses an enzyme which can deiodinate diiodotyrosine when it is present as the free amino acid but not when it is bound in peptide linkage The optimum pH for this reaction is about 6 During the course of a tryptic digestion of thyroglobulin diiodotyrosine will be liberated and the pH may fall

attempt to alter the other end of the molecule, i e the diiodophenol group The first compound prepared with this end in view was 3 5 3 triiodo thyronine obtained by iodinating 3 5 diiodothyronine in ammoniacal solution with two atoms of iodine only



Two dimensional chromatography in butanol acetic acid and butanol dioxan ammonia showed that triiodothyronine and Unknown 1 had identical R_F values in these solvent systems[841] and it was therefore highly probable that the two were identical (see Fig 1) It therefore became essential to isolate triiodothyronine from the thyroid gland to complete the identification This was finally achieved in the following way Fresh-frozen ox thyroid was partially defatted minced and hydrolysed in 5 kg batches in ammonium chloride ammonia urea buffer with powdered trypsin at 37°C The hydrolysate was then brought to pH 4 with hydrochloric acid and chilled The precipitate which separated was collected and while wet was extracted repeatedly with chloroform to remove tarry products and fat It was then dissolved in 2N-NaOH and extracted twice with *n* butanol The butanol extracts were concentrated to dryness the residue was dissolved in water and brought to pH 3–4 with hydrochloric acid The precipitate thus obtained was collected washed with chloro form and petrol and dried Repeated ether extraction of the dried material gave a powder which contained much thyroxine in order to remove most of the latter the powder was extracted with boiling 2N hydrochloric acid and the filtrate from undissolved thyroxine was neutralized with alkali The resulting precipitate however still contained thyroxine Final purifi cation was effected by separating thyroxine from triiodothyronine on a kieselguhr column and eluting with chloroform butanol NaOH[841 843]

The triiodothyronine containing fraction was concentrated to dryness dissolved in water and brought to pH 4 with a mixture of hydrochloric and acetic acids A final purification was effected by dissolving the amino acid in boiling 2N HCl On slow cooling the hydrochloride separated in spear shaped needles which had a melting point of 201–203 C and also decomposed at this point The mixed melting point with synthetic L triiodothyronine hydrochloride was not depressed the natural hydro chloride had I 55 4% $C_{15}H_{12}O_4NI_3$ HCl requires I 55 4%

Crystallographic examination of the free amino acid and of its hydro chloride[817] also showed that it was identical with synthetic 3 5 3 - triiodo L thyronine

While this work was in progress Roche *et al* [1337 1338] simultaneously

Two syntheses of monoiodotyrosine have been described The first, by Harington and Pitt Rivers[697] consists of mononitration of tyrosine followed by reduction to 3 aminotyrosine This is converted to 3 iodo tyrosine by diazotization and treatment with potassium iodide in acid solution Unfortunately this method has not proved useful in the hands of other workers owing to a faulty description of the isolation of monoiodo tyrosine the step involving neutralization with ammonia of an aqueous sulphuric acid solution of monoiodotyrosine has been accidentally omitted from the description and without this addition the amino acid does not separate even from the concentrated solution

A simple preparation of monoiodotyrosine has also been described by Pitt Rivers[1 5] This merely consists of the iodination of tyrosine in ammoniacal solution the product is always contaminated with a little diiodotyrosine, but is adequate for chromatographic identification of biological materials

TRIIODOTHYRONINE

The work of Leblond Gross and colleagues[637 638 639] was the starting point for a systematic investigation of new iodinated compounds in the thyroid These workers administered ^{131}I to rats by subcutaneous injection and killed the animals at various time intervals thereafter The thyroid glands were removed, homogenized and extracted with *n* butanol The concentrated butanol extracts were analysed with carrier thyroxine diiodo and mono iodotyrosines and iodide by two dimensional chromatography Separation of the iodine containing compounds was effected first with butanol saturated with 2N acetic acid and then with butanol dioxan 2N ammonia (4 1 5) All the carriers corresponded with radioactive spots on the chromatograms revealed by autoradiography Besides the known compounds three radioactive spots appeared which did not correspond with any known compounds One of these compounds named Unknown 1 had an R_F value identical with that of thyroxine in butanol acetic acid and very similar to it in butanol dioxan ammonia Further Unknown 1 was the only iodinated compound besides thyroxine and iodide to be found in the blood of these animals Gross and Pitt Rivers[640] later showed that Unknown 1 was present in the plasma of patients who had been treated with therapeutic doses of radioiodine and the identification of Unknown 1 was therefore attempted The similarity between the R_F values of thyroxine and the unknown compound suggested that they were probably structurally related and a number of derivatives of thyroxine synthesized by Harington and co workers[57 58 255] were used as carriers on chromatograms containing Unknown 1 None of these corresponded with the unknown compound These thyroxine derivatives all possessed modified side chains Failure to identify Unknown 1 with any of them led to an

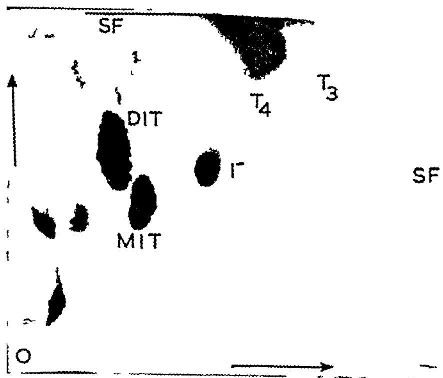


Fig 1

Autoradiogram of two dimensional chromatogram of radioactive products of hydrolysis of rat thyroid tissue 16 hr after injection of a tracer dose of ^{131}I Solvent systems vertical *n* butanol saturated with 2N acetic acid horizontal *n* butanol dioxan 2N NH (4 1 5) O = origin SF = solvent front T_4 = thyroxine T_3 = triiodothyronine MIT = monoiodotyrosine DIT = diiodotyrosine I^- = iodide other spots unknown (from Gross and Pitt Rivers unpublished)

reported the synthesis of 3 5 3 triiodothyronine from 3 5 diiodothyronine and its chomatographic detection in rat thyroid hydrolysates after the administration of ^{131}I Since that time the presence of triiodothyronine in rat thyroids has been confirmed [09 949], it has been found in the thyroids of humans[209], a teleost (*Periophthalmus koelreuteri*), *Protopterus annectens* Owen[946 947] and the domestic fowl[1457] Triiodothyronine has again been demonstrated in human blood[146] and in blood from the thyroid vein of the sheep horse and calf[1592]

Some physical properties of thyroxine and related compounds are given in Appendix B

Other Iodinated Thyronines

Two other iodinated thyronines[1368] have been detected in rats' thyroids with the aid of radioactive iodine and chromatography, namely 3 3 -diiodothyronine and 3 3 5 triiodothyronine, the former but not the latter has also been detected in the blood Roche *et al* [1357] have also identified these compounds in pigs thyroid, and have characterized them by a number of colour reactions Both these compounds appear to be formed in very small amounts, and their existence is as yet unconfirmed.

Biological Significance of the Iodothyronines

Before proceeding to a discussion of the biosynthesis of the thyroid hormone it would be as well to pause to consider which products of the thyroid exhibit physiological activity Soon after the discovery of 3 5 3 -triiodothyronine it was found to possess even greater activity than thyroxine in preventing thiouracil induced goitre in rats and in stimulating oxygen consumption in laboratory animals and in hypothyroid humans Detailed references to the mass of work which has been done on the potency of 3 5 3 triiodothyronine *in vivo* will be found in Chapter 5 It is only reported here to show that 3 5 3 -triiodothyronine by virtue of its activity, should be included with thyroxine as a thyroid hormone

If we now consider the isomeric triiodothyronine and 3 3 diiodothyronine, their inclusion among the thyroid hormones cannot at present be justified 3 3 5 -Triiodothyronine is virtually inactive[1365] in the goitre prevention assay and in tadpole metamorphosis tests the activity of 3 3 -diiodothyronine is at present debatable Roche and co workers[1365] claim a relatively high potency for this compound of the order of 70–80% that of thyroxine Stasilli *et al* [1512] Brownstone and Pitt Rivers (unpublished) and Halmi (personal communication), on the other hand have found little or no antigoitrogenic activity by the goitre prevention method when the compound was given in amounts 100 times greater than a highly active dose of thyroxine The discrepancy here may be due to the fact that the 3 3 -diiodothyronine originally used by Roche and co-workers

was prepared in such a way that the presence of small amounts of the highly active 3 5 3 -triiodothyronine could not be excluded, while the material used by Brownstone and Pitt Rivers was synthesized by a method* in which no possibility of 3 5 3 -triiodothyronine formation existed

Because of the above considerations the discussion on the biosynthesis of the thyroid hormones will be confined to thyroxine and 3 5 3 triiodothyronine

Other Iodinated Amino Acids

Monoiodohistidine was chromatographically identified by Roche *et al* [1340] in ^{131}I labelled rat thyroid hydrolysates, but the amounts detected were small Recently Block *et al* [181] have shown by similar methods that diiodohistidine is also present in the thyroid The physiological significance of these compounds is not known

* 3 3 Diiodothyronine was synthesized by Meltzer and Stanaback (personal communication) in the following manner α benzamido-3 nitro-4-(4 methoxyphenoxy) cinnamic acid was reduced to the 3 amino compound diazotized and converted to the 3 iodo-compound demethylation and hydrolysis led to 3 iodothyronine which on iodination gave 3 3 -diiodothyronine Roche *et al* [1358] have recently published a similar synthesis of 3 iodothyronine

(I) FORMATION OF THYROXINE FROM DIIODOTYROSINE *in vitro*

The first experimental support for Harington s suggestion that thyroxine was formed from diiodotyrosine in the thyroid gland came from the work of Ludwig and von Mutzenbecher[991] in 1939, these authors showed that when casein is iodinated in a mildly alkaline buffer the resulting iodo protein yields on hydrolysis thyroxine together with mono and di iodo tyrosine In the same year von Mutzenbecher[1134] showed that when diiodotyrosine was incubated at 37°C at pH 10 small amounts of thyroxine were obtained These unexpected findings were soon confirmed[695 180 99] and have formed the basis of extensive studies on the production of arti ficial iodoproteins with high biological activity[1286 1230 1284 1344] the practical importance of these proteins is mentioned in Chapter 5

The mechanism of the conversion of diiodotyrosine to thyroxine was first studied by Johnson and Tewkesbury[816] in a brief report these authors claimed that the side chain which is split off during the reaction appeared in the reaction medium as pyruvic acid but did not state the method of identification used Harington[693] presented a theoretical model for the oxidative conversion of diiodotyrosine to thyroxine and later showed with Pitt Rivers[698] that the reaction was indeed an oxidative one and did not proceed in the absence of oxygen or in the presence of thio sulphate the rate of conversion was accelerated by the action of oxidizing agents such as peroxide and iodine

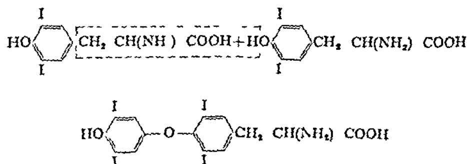
Later Pitt Rivers[12 0] showed that protection of both the amino and carboxyl groups of diiodotyrosine led to greatly increased yields of the thyroxine derivative thus while diiodotyrosine itself only gave 4% of thyroxine (calculated on the amount of diiodotyrosine lost during the reaction) *N* acetyldiiodotyrosine gave a net yield of 25% of *N* acetyl thyroxine and *N* acetyldiiodotyrosylglutamic acid gave 35% of *N* acetyl thyroxylglutamic acid In these studies an unsuccessful attempt was made to determine the nature of the side chain lost during the reaction

Sela and Sarid[1440] have studied the formation of thyroxine during the iodination of poly L tyrosine and have shown that serine is also formed during the reaction no quantitative relationship between thyroxine (and triiodothyronine) on the one hand and serine on the other was established Recently Pitt Rivers and James[1229] have shown that during the aerobic incubation of the acetyldiiodotyrosyl peptide α *N* acetyl ε (*N* acetyl diiodotyrosyl)lysine α *N* acetyl ε (*N* acetylthyroxyl)lysine is formed in yields as high as 50% net at the same time acetamide and ε hydroxy pyruvoyl α *N* acetyllysine (isolated as the *p* bromophenylhydrazone) are formed in amounts molecularly equivalent to the amount of the thyroxine derivative obtained

BIOSYNTHESIS OF THE THYROID HORMONES

It has been known for many years that the thyroid gland concentrates iodide from the blood and converts it to organically bound iodine As long ago as 1914 Blum and Grutzner[163] showed that the thyroid of the dog increased its iodine content after the administration of potassium iodide and retained the stored iodine for a considerable time Marine and co workers[10 7, 10 9] found that the thyroids of dogs concentrated iodide when perfused with potassium iodide both *in vivo* and *in vitro* hyperplastic glands concentrated more iodide than normal glands The uptake of iodide *in vitro* depended on the tissue being viable and was inhibited by cyanide maximal uptakes were observed when the concentration of iodide in the perfusate was low These authors showed that most of the iodine taken up by the glands could not be removed by perfusion Blum[162] suggested that the thyroid contains an iodase which converts iodide to iodine and thereby enables it to be incorporated in organic linkage This belief is still held today The bound iodine first appears in the iodotyrosines and later in thyroxine and triiodothyronine

Harington[339] suggested that thyroxine was derived in the thyroid by the coupling of two molecules of diiodotyrosine and the loss of an alanine side chain



As we shall see this theory has since received much support both from experiments *in vitro* and *in vivo*

containing 14 mg of sodium iodide The results obtained can therefore have only a qualitative significance, and do not reflect the physiological processes taking place when iodine is taken up by the thyroid gland

Perlman *et al*[120] first demonstrated the profound difference between the uptake of a tracer dose of radioactive iodide (^{131}I) and the uptakes of doses to which different amounts of stable iodide were added In rats given tracer doses 65% of the administered dose was found in the thyroid 1–2 days after the injection when 30 μg ^{127}I was added to the ^{131}I, the uptake was reduced to 7% of the administered dose and was reduced still further (to 2%) when 500 μg ^{127}I was given Perlman *et al*[1203] further studied the incorporation of tracer doses of ^{131}I into sheep and rat thyroids *in vivo* and identified labelled thyroxine like and diiodotyrosine like compounds obtained by butanol fractionation In the thyroids of both species the ^{131}I in the diiodotyrosine fraction exceeded that in the thyroxine fraction at all times but the thyroxine fraction increased with time Mann *et al*[1019] administered ^{130}I to dogs in tracer doses and analysed the thyroids at different time intervals after the dose for total ^{130}I and ^{127}I and for ^{130}I and ^{127}I in the inorganic and thyroxine like fractions their results were in agreement with those of Perlman *et al*[120] and they concluded that diiodotyrosine is the precursor of thyroxine in the thyroid gland

Later Morton and Chaikoff[1115] investigated the uptake of ^{131}I in rat dog and sheep thyroid slices in a buffer containing small amounts of stable iodide (0 1 μg/ml) and found that these slices incorporated iodide into diiodotyrosine and thyroxine the slices from rat thyroids were the most active

Since this early work the presence of monoiodotyrosine and triiodothyronine in the thyroid has also been demonstrated (see Chapter 2) At this stage therefore we may propose a scheme whereby iodine is metabolized in the thyroid gland

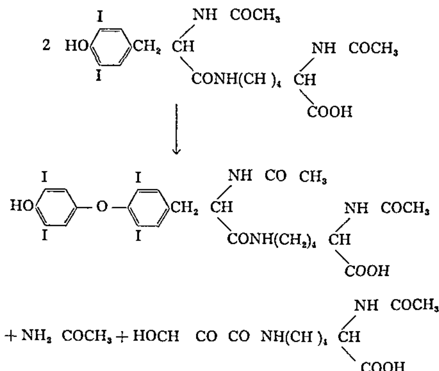
(a) The thyroid gland concentrates iodide from the circulation

(b) Iodide is oxidized to iodine and incorporated into tyrosine molecules first as monoiodotyrosine

(c) Further iodination of monoiodotyrosine yields diiodotyrosine

(d) Coupling of two molecules of diiodotyrosine leads to the formation of thyroxine

(e) Triiodothyronine can be synthesized by two pathways (i) by a coupling similar to that involved in the formation of thyroxine in which one molecule of monoiodotyrosine and one molecule of diiodotyrosine take part (ii) by a partial deiodination of thyroxine



It is not known whether either serine or hydroxypyruvic acid are formed during the biosynthesis of thyroxine in the thyroid gland, but either compound would be a likely product of the 3 carbon side chain which is lost during this reaction It is not intended to suggest that the mechanism of formation of thyroxine *in vitro* necessarily represents a model for thyroxine *formation in the thyroid gland*

(II) Biosynthesis of the Thyroid Hormones *in vivo* Studied with Radioactive Isotopes of Iodine

Early experiments on iodine metabolism in the thyroids of experimental animals with radioisotopes of iodine were made with ^{128}I which has a *half life of 25 min*[732, 733, 930, 928] Ariel *et al*[50] used *mixtures of* ^{128}I and ^{130}I (half life 12 5 hr) in their studies in rabbits The isotope of iodine ^{131}I which has a half life of 8 days was first used by Hamilton and Soley to investigate iodine metabolism in normal humans and in patients with hypo and hyper thyroidism and various types of goitre In all these studies quoted above large doses of stable iodide (^{127}I) were given with the radioactive isotope thus Leblond *et al*[930] gave 750 μg stable iodide to rats Hertz *et al*[733] gave 5 mg of stable iodide to rabbits and Hamilton and Soley[471, 472] administered ^{131}I to humans in solutions

methods Dziemian[415] showed that extracts of the protease could be obtained from rat and guinea pig thyroid glands by 60% glycerol water extraction such extracts hydrolysed edestin at pH 4 and the proteolytic activity was enhanced by cysteine Kamner *et al*[830] found that dog thyroids could liberate tyrosine from haemoglobin in aqueous acetic acid at pH 3 55, Weiss[1705] showed that beef thyroid had a peptidase activity at pH 7 2–8 4 and a protease activity which was maximal at pH 4, proteolytic activity disappeared in the physiological pH range Roche *et al*[1334] showed that extracts of ^{131}I labelled thyroid from the dog and pig liberated ^{131}I iodotyrosines and thyroxine when incubated at 37°C and pH 3 5 Trikojus and co workers[1062, 1061] have obtained the most highly purified preparations of thyroid protease and have shown that it possesses peptidase activity in the same pH range as pepsin Alpers and colleagues[59, 37] have also partially purified the thyroid protease by phosphate precipitation of saline extracts of rat thyroid and have demonstrated its ability to hydrolyse thyroglobulin by ultracentrifugal analysis ^{131}I labelled thyroglobulin was shown to yield non dialysable fractions as well as thyroxine the iodotyrosines and iodide The enzyme preparation was active over a pH range between 2 5 and 5 5 with maximum activity at pH 4 9 Clements[90] has demonstrated proteolytic activity in the thyroid of the dogfish (*Scyliorhinus canicula*) and has found that glands from female fish were more active than those from male fish (see Leloup[945])

Under normal conditions thyroxine and triiodothyronine are the only iodinated amino acids to be secreted into the blood neither monoiodotyrosine nor diiodotyrosine escapes from the thyroid gland Roche Michel and colleagues[1355, 1342] demonstrated some years ago that thyroid slices were able to deiodinate free mono and diiodotyrosine but had no action on these amino acids when they were bound in thyroglobulin further these workers were unable to demonstrate any action of the thyroid deiodinase on thyroxine or triiodothyronine For this reason they concluded that triiodothyronine could not arise in the thyroid gland by partial deiodination of thyroxine but must be formed by the coupling of one molecule of monoiodotyrosine with one molecule of diiodotyrosine Recently Tata (unpublished) has found that pig thyroid deiodinase deiodinates thyroxine readily if it is free from blood proteins However triiodothyronine has not been detected The iodotyrosines are not bound to these proteins and are therefore liable to attack by the deiodinase the iodide thus liberated is presumably recycled in thyroid hormone synthesis

In normal thyroid tissue the iodide that enters the gland is almost immediately bound in organic linkage although some iodide is always present and indeed may return to the circulation without undergoing organification[1140, 791] The appearance of labelled iodinated amino acids in thyroid tissue after injection of ^{131}I is shown in Fig 2 It will be seen

Both mechanisms may lead simultaneously to the formation of triiodo thyronine in the gland Evidence for and against these two pathways will be discussed later

Very little is known about the enzymes responsible for these reactions It has been postulated that the conversion of iodide to iodine could be mediated by a thyroid peroxidase and Dempsey[364] has obtained histo chemical evidence of peroxidase activity in the thyroid cells De Robertis and Grasso[371] also described peroxidase activity in both cells and colloid of rat tissue Glock[588] was unable to isolate peroxidase or demonstrate its presence in thyroid tissue however it has been pointed out by Astwood[70] that the amount of peroxidase required to oxidize amounts of iodide in thyroid tissue suitable for manometric estimations is so small that any quantitative assessment would be virtually impossible Keston[853] showed that iodination of tyrosine in casein could be brought about with iodide after the addition of flavine oxidase Both Weiss[1704] and Fawcett and Kirkwood[456] claimed that cell free thyroid preparations to which copper had been added could iodinate free tyrosine from iodide but not tyrosine bound in protein in both reports the amounts of copper used were very large and the more recent work of Taurog and his colleagues[1587, 1630] using a particulate fraction from sheep s thyroids consisting of mito chondria and microsomes has demonstrated that iodination takes place on bound tyrosine molecules in the absence of added copper further, the addition of flavine co factors greatly enhanced this iodinating system The pH optimum both in the presence and absence of flavine co factors was between 9 and 10 it was suggested[1630] that this might be due to increased ionization of the phenolic group at the high pH or to the formation of some active form of iodine but the possibility was not excluded that this pH was the optimum for iodide oxidizing activity of the enzyme

It is not yet known whether iodination in the thyroid gland occurs when tyrosine is free or when it is bound in the protein but it is likely from the work of Taurog and co workers cited in the previous paragraph that mono- and diiodotyrosine are formed from tyrosine bound in the thyro globulin molecule Neither is it known whether the coupling reaction of diiodotyrosine to form thyroxine occurs with free or bound diiodotyrosine the coupling of diiodotyrosine derivatives *in vitro*[120, 129] which occurs very readily suggests that thyroxine is formed from bound diiodotyrosine in the gland

Most of the thyroidal iodine is stored in thyroglobulin and the latter must be hydrolysed before the hormone can be secreted into the circulation (see Chapter 4) The existence of a thyroid protease was first demon strated by histochemical methods by De Robertis[369] in the colloid from single follicles of rat thyroid De Robertis and Nowinski[372] demonstrated protease activity in normal and pathological human thyroids by similar

other workers Hogben[748] and Spaul[1507] showed that metamorphosis of axolotls was precipitated by the administration of anterior pituitary extracts Uhlenhuth and Schwartzbach[1656] made similar observations in other species of salamanders and found that treatment with anterior pituitary extracts was followed by hyperplastic changes in the thyroid Smith[1493] clearly demonstrated atrophy in the thyroid of the rat after hypophysectomy and showed that this could be reversed by pituitary transplants Aron[51] administered pituitary extracts to guinea pigs and observed hypertrophy of thyroid epithelium increase in thyroid weight and massive secretion of colloid, this stimulation could be reversed by simultaneous administration of thyroxine[52, 53] Eitel *et al* [425] and Closs *et al* [292] found that the thyroidal stimulation by pituitary extracts in guinea pigs was accompanied by a decrease in its iodine content. Schockaert and Foster[1429] also reported depletion of thyroidal iodine after administration of pituitary extracts in ducks

The compensatory hypertrophy of the rat thyroid observed by Logothetopoulos and Doniach[983] after partial thyroidectomy probably results from the increased secretion of thyroid stimulating hormone (thyrotrophin TSH) which follows the temporary fall in circulating thyroid hormone level Fontaine[511] has shown that after thyroidectomy in rats the plasma level of TSH rises and that of the pituitary falls these changes are directly proportional to the amount of the thyroid gland removed Fontaine concludes that this has a bearing on compensatory hypertrophy of the thyroid after partial thyroidectomy

It is evident therefore that the thyroid and anterior pituitary glands control each other by what has been described as a feedback mechanism in the healthy animal this insures an adequate but not excessive amount of thyroid hormone for the regulation of metabolic processes[344, 314, 1685]

It has been suspected for a long time that the pituitary must play some part in Graves disease The similarities seen in thyroid function in thyrotoxicosis and in animals treated with TSH are very striking but neither changes in the pituitary nor increased levels of circulating TSH have been demonstrated in hyperthyroid patients[691, 752, 1064, 1271] The role of the pituitary in the aetiology of hyperthyroidism remains to be established although the work of Sonenberg *et al* [1505] on the inhibition of normal and pathological thyroid function by partially acetylated TSH suggests that hyperactivity of the anterior pituitary may be a causal factor in Graves disease

The effects of hypophysectomy on the thyroid have also been recognized for many years Paulesco (see Cushing[341]) showed that hypophysectomy in dogs resulted in symptoms similar to those described by Kocher[870] after removal of the thyroids of goitrous patients Cushing[341] observed involution of the thyroid in hypophysectomized dogs Hypophysectomy

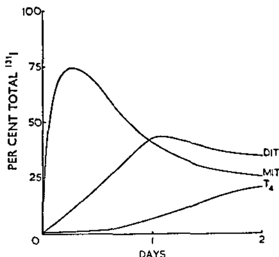


Fig 2

Distribution of ^{131}I in the principal iodinated amino acids of hydrolysed rat thyroid gland in function of time after the administration of ^{131}I MIT = monoiodotyrosine DIT = diiodotyrosine T_4 = thyroxine

that monoiodotyrosine first appears, and that as it declines the amount of diiodotyrosine increases similarly thyroxine is formed at the expense of diiodotyrosine Hence monoiodotyrosine is the precursor of diiodotyrosine and diiodotyrosine the precursor of thyroxine The precursor product relationship between diiodotyrosine and thyroxine foreseen by Harington[689] was indirectly demonstrated by Mann *et al* [1019] and later by Taurog *et al* [1585]

(III) Factors that Influence Thyroid Function

(*a*) *The Pituitary Thyroid Relationship*

It is impossible to understand the work that has been done towards the elucidation of the nature of thyroid hormone biosynthesis without first considering the intimate relationship between the thyroid and pituitary glands In many of these studies the effects of hypophysectomy have been examined in conjunction with the effects of antithyroid drugs and a brief description of the mutual control of these two glands is therefore necessary

More than 100 years ago Nièpce[1157] observed that the anterior pituitary glands of cretins and goitrous humans and animals were markedly hypertrophied Later Rogowitsch[1372] showed that thyroidectomy also resulted in anterior pituitary hypertrophy, this hypertrophy could however[980] be reversed by administration of thyroid substance The effects of anterior pituitary extracts on the thyroid glands of animals has been studied by

Brown Grant[232] has postulated an extension to the feedback mechan ism whereby the hypothalamus controls secretion of TSH by the pituitary by removing thyroid hormone from the arterial blood supply to pituitary He argues that pituitary stalk section would destroy the portal blood supply, which would result in a higher thyroid hormone concentration in the anterior pituitary and a consequent decrease of thyroid activity on the other hand stimulation of the hypothalamus would be followed by increased blood flow and withdrawal of thyroid hormone from the pituitary which would result in increased TSH secretion and thyroid activity

It is clear from the findings discussed above that the secretion of TSH (and trophic hormones) from the anterior pituitary is influenced by the hypothalamus the actual mechanism of this control is not conclusively established

(*b*) *Dietary Factors*

(i) *Iodine*—Before discussing the biosynthesis of the thyroid hormone *in vivo* it is necessary to consider the effects of certain nutritional factors that influence the thyroid gland As we have already seen (Introduction) Chatin attempted more than 100 years ago to associate endemic goitre in certain parts of Europe with the iodine content of water and foodstuffs doubts were however cast on his work and for many years the relationship between iodine deficiency and goitre was ignored

The extensive studies of Marine and his colleagues on iodine prophylaxis and goitre in Ohio reawakened interest in the subject and were followed by similar investigations by Remington in various parts of America by Klinger and von Fellenberg in Switzerland and by Hercus in New Zealand (see [105 1178 691]) The relationship between iodine deficiency and goitre is described in Chapter 9 and will not be considered further at this point It is now generally accepted that the level of dietary iodine is of paramount importance for the proper working of the thyroid gland At the present time Greenwald[617 618] is one of the few who contends that endemic goitre and iodine deficiency are unrelated

In animals iodine deficiency also results in thyroid hyperplasia Remington and co workers[957 958] have related iodine intake to goitre in rats and devised a low iodine diet which is much used today for studies in iodine deficiency in rats This diet contains only 15 μg of iodine per kg and affords a daily intake of 0 14 μg of iodine per rat Remington and colleagues showed that a diet containing 265 μg of iodine per kg prevented the development of goitres in their animals and caused the regression of goitres already present It has been suggested that the Remington diet contains an active goitrogen this problem has recently been investigated by Axelrad *et al* [81] None of the constituents was found to be goitrogenic except chloride when this was omitted from the diet a pronounced

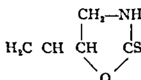
in tadpoles has been shown to prevent metamorphosis[149, 31] In rats[321] *hypophysectomy results in a fall in the BMR (basal metabolic rate)* a shortening of the life span[1494] and a loss of activity[130] In man, the absence of pituitary function is followed by symptoms which include those of hypothyroidism[1465, 464, 952, 1064] This syndrome does not concern us in the present discussion and the effects of hypophysectomy will be considered in the following section in so far as they affect thyroid hormone biosynthesis

Some mention must be made of the role of the hypothalamus in TSH secretion by the pituitary Pituitary stalk section has been repeatedly shown[1659, 366, 2, 1, 123, 704-6, 708, 232] to interfere with thyroid function Greer[621, 62, 624] has found that bilateral electrolytic lesions in the hypothalamus of the rat interfered with the hyperplastic response of the thyroid to the feeding of thiouracil but did not interfere with the iodide concentrating mechanism He therefore postulated the existence of two anterior pituitary secretions one which stimulated the growth of thyroid follicular cells and the other which stimulated iodide concentration Halmi and colleagues[663, 192] have also studied the effects of hypophysectomy and bilateral hypothalamic lesions on thyroid function in rats but find no necessity for postulating two thyroid stimulating hormones VanderLaan and Caplan[1667] support Halmi *et al*[663] in this contention rather than Greer[622]

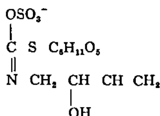
Early work on the effect of hypothalamic stimulation on thyroid function has been critically reviewed by Harris[705] and will not be discussed Prolonged electrical stimulation of the hypothalamus was shown by Colfer[97] to produce histological signs of increased thyroidal activity in rats and rabbits but Green and Harris (see [95]) could not obtain any evidence of a rise in BMR in rabbits that had received such hypothalamic stimulation, however they considered that the determination of BMR in rabbits might not be a sensitive enough index of thyroid activity to warrant any conclusions Harris and Woods[707] have shown that electrical stimulation of the hypothalamus usually results in a depression of ^{131}I release from the thyroid glands of normal rabbits in adrenalectomized rabbits maintained on cortisone stimulation of the hypothalamus (tuber cinereum) caused a marked increase of thyroidal secretion of ^{131}I the authors have explained these results as follows in the normal rabbit stimulation of the tuber cinereum enhances the liberation of adrenocorticotrophic hormone (ACTH) from the pituitary gland and thereby increases the concentration of adrenal steroids in the blood these tend to inhibit the release of TSH from the pituitary and thyroid function is depressed In the adrenalectomized rabbit maintained on cortisone stimulation of the hypothalamus cannot result in an increase of circulating adrenal steroids but will result in an increase in TSH secretion with a consequent rise in thyroid activity

The nature of the *Brassica* goitrogens has also been studied Mustard oils or isothiocyanates compounds characteristic of the *Brassica* genus, were first considered Marine *et al*[1026] tested several of them in rats but found that none exhibited any goitrogenic properties Organic cyanides, also important constituents of *Brassicae*, were however found to be goitro genic though curiously enough thiocyanate was not Richter and Clisby[1301] discovered that phenylthiourea was goitrogenic in rats and shortly afterwards Kennedy[848] suggested that allylthiourea might be a natural goitrogen

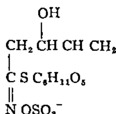
In 1949, Astwood *et al*[72] isolated an antithyroid compound from the yellow turnip (rutabaga) Astwood and his colleagues showed that this was the cyclized derivative of an isothiocyanate L-5 vinyl 2 thiooxazolidone



The vinylthiooxazolidone does not exist as such in the yellow turnip it exists in a combined form from which it is liberated by enzymic action[68, 60] If the turnip or other vegetables containing this compound are cooked their goitrogenic activity is destroyed Greer[623] has recently isolated the pre cursor (progoitrin) of L 5 vinyl 2 thiooxazolidone (goitrin) and has shown it to be a glucoside related to L 2 hydroxy 3 butenyl isothiocyanate he has attributed to it the structure



However the work of Ettlinger and Lundeen[458] on the structure of the mustard oil glucosides sinigrin and sinalbin proves that the structure of progoitrin must be



decrease in thyroid weight was observed the goitrogenicity of large amounts of chloride in the rat has since been established[801] Chapman[276] demonstrated marked hypertrophy in the thyroids of rats maintained on a low iodine diet for 55 days Leblond and Mann[9 7] also found hyperplastic changes in rats thyroids on a low iodine diet for 70 days and these glands showed a high ^{131}I uptake Maloof *et al*[1017] observed an increased ^{131}I uptake and thyroid size in rats that had only been on a Remington diet for 11 days Money *et al*[1098] found that enlargement of rat thyroids did not appear for more than 3 months on a low iodine diet although increased ^{131}I uptake in these animals appeared very soon after the diet was started Halmi[659] also was unable to detect morphological evidence of thyrotrophic activation of the thyroids in rats after 19–20 days on a Remington diet but found that the thyroid/serum iodide ratios in these animals were much higher than those in animals fed on a high iodine diet

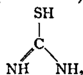
(*ii*) *Goitrogens in food*—Diets rich in protein fat and carbohydrate and deficient in vitamins have all been held responsible for the development of goitres these factors cannot however be shown to affect biochemical and physiological function of the thyroid in a direct manner, and will not be discussed here The reader is referred to the review by Greer[620] for a description of this work

The recognition that certain foodstuffs interfere with iodine metabolism in the thyroid stems from the discovery by Chesney *et al*[281] that rabbits fed exclusively on cabbage developed enormous goitres These findings were soon confirmed in other laboratories and other foodstuffs were shown to possess goitrogenic properties these included a number of *Brassicae* such as cauliflower brussels sprouts and turnips[1025 10 6] Hercus and Purves[726] found that the seeds of certain *Brassicae* notably those of cab bage rape and mustard were goitrogenic when fed to rats and they reported goitres in sheep fed on large amounts of turnip Cabbage goitre in rats was found to be reversible by the administration of iodide[1700 1051], and soya bean goitre in rats was also reversed by iodide[51] however, McCarrison[1051] and Kennedy and Purves[849] could not entirely reverse soya bean or *Brassica* goitre in rats with iodide

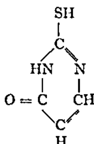
The cabbage goitre of Chesney *et al*[281] differs from the goitre that follows the ingestion of *Brassica* seeds in that the former is reversible by administration of iodide while the latter is not it can only be reversed by administration of thyroxine or related compounds These natural goitrogens have a similar action to that of the antithyroid drugs (see Section *C*) and interfere with organic binding of iodine in the thyroid[154] A most significant finding on the mode of action of these goitrogens was made when Griesbach *et al*[626] reported that hypophysectomy prevented the development of goitres in rats fed rape

that the drugs interfered in some way with thyroid hormone synthesis

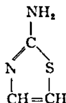
This work immediately suggested the possible usefulness of the anti thyroid drugs in the control of hyperthyroidism in man and in the following years an enormous amount of work was done to find antithyroid agents with the maximum therapeutic effect and the minimum toxicity This work has led to the discovery of the activity of the substituted thiouracils and other thiocarbamide derivatives (aminothiazoles and mer captoimidazoles)



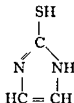
Thiourea (thiocarbamide)



2 Thiouracil



2 Amino thiazole



2 Mercap-toimidazole

For reviews on the antithyroid drugs the reader is referred to Astwood[67, 69] McGinty[1057], Trotter[1636] VanderLaan and Storrie[1669] Astwood and Solomon[74]

At first no clear differentiation was seen between the mode of action of thiocyanate on the one hand the thiocarbamides on the other but the fact that thiocyanate action could be reversed by administration of iodide while the action of the thiourea derivatives could only be reversed by thyroxine demonstrated that the two types of drugs acted at different levels of thyroid hormone biosynthesis These properties of the antithyroid drugs have been used to study independently the concentration of iodide by the thyroid and its incorporation into thyroglobulin (section V) Neither thiocyanate nor perchlorate completely inhibits iodide uptake by the thyroid but the amount of iodine which gets into the gland probably represents that which enters by simple diffusion organic binding is not inhibited and this small amount of iodide has recently been shown to be incorporated into the iodotyrosines and thyroxine[1, 27] When the binding of iodine is blocked by propylthiouracil the thyroid serum iodide ratio increases to many times the normal value and all the iodine in the gland is present as iodide [1058, 1670, 1584] This effect is abolished by hypophysectomy[1668]

The inhibition of thyroid hormone synthesis in the thyroid results in a decrease and finally a disappearance of circulating thyroid hormone as a result of this the brake on the pituitary is removed and it secretes an increased amount of thyrotrophin this in turn produces the hyperplastic changes in the thyroid The degree of inhibition of thiouracil induced

Clements and Wishart[289] have shown that in Tasmania and certain parts of Australia, the milk of cows fed the *Brassica* chou moellier contains an active goitrogen Goitres developed in schoolchildren drinking this milk and were not prevented by the weekly administration of 10 mg potassium iodide further calves fed on chou moellier showed marked thyroidal hyperplasia and extracts of chou moellier produced goitres in rats and interfered with ^{131}I uptake by the thyroid Wright[1753] has recently demonstrated that the milk of animals fed kale contains a thiocyanate type of goitrogen this milk was able to discharge ^{131}I from the thyroid glands of rabbits pre treated with propylthiouracil further it contained 4 6 mg thiocyanate per 100 ml compared with 0 8 mg per 100 ml in milk from cows fed on pasture

The goitrogenic activity of groundnut (peanut *Arachis hypogaea*)[1514 1124] (see *Nutr Rev* 16 19 (1958)) and other foodstuffs[625 627] has also been studied Although many of the foods tested by Greer and Astwood[625] possessed only doubtful goitrogenic activity there is no doubt that certain vegetables notably the *Brassicae* and groundnut do contain potent goitrogens and Astwood s[68] statement that some other factor besides iodine deficiency is contributing to goitrogenesis has been confirmed

(*c*) *The Antithyroid Drugs*

The first observation of drug interference with thyroid function was that of Barker[100] who found that goitres developed in two patients who were given prolonged treatment with thiocyanate for hypertension Barker showed that these goitres could be reversed with thyroxine but Astwood[66] later showed that they could be reversed by simultaneous administration of iodide this meant that thiocyanate must inhibit thyroidal uptake of iodine at the level of iodide concentration Certain anions notably perchlorate have also been shown to inhibit iodide concentration by the thyroid of the rat[1757 1 58] and man[1527] and have been used to control hyperthyroidism in man[591 1107]

In 1941 MacKenzie *et al*[1001] reported briefly that sulphaguanidine was goitrogenic in rats Two years later MacKenzie and MacKenzie[1000] and Astwood *et al*[75] simultaneously described the effects of some sulphonamides and thioureas on the thyroids of certain animals hyperplastic changes were seen in the thyroids of rats mice and dogs but chicks and guinea pigs were not affected Further the rat was shown to exhibit symptoms of hypothyroidism such as a decreased food intake growth and oxygen consumption The thyroid hyperplasia from these drugs was not reversed by the administration of large amounts of iodide but was reversed by thyroxine and by hypophysectomy Chaikoff and colleagues[526 516] showed that thiouracil depressed the formation of thyroxine from diiodotyrosine by the rat thyroid *in vivo* and *in vitro* it was concluded

The quantitative relationship between cold and thyroid stimulation was clearly demonstrated by Dempsey and Astwood in 1943[365] Rats were given thiouracil in their drinking water and the daily amount of L thyroxine required to prevent thyroid hyperplasia at 25°C, 1°C and 35°C was determined at 25 C, this amount was found to be 5 μg The requirement rose to 9 5 μg in the animals kept at 1°C and fell to 1 7 μg in the animals kept at 35°C

It has been repeatedly shown that moderate exposure of rats to cold stimulates ^{131}I uptake by the thyroid[925 193 1724] and increases ^{131}I dis charge from the gland[233 229 1752] It has also been shown that short term exposure to cold is more effective in stimulating the thyroid than pro longed exposure (see references above also [866]) It has been suggested that moderate exposure to cold probably causes an increased secretion of TSH by the anterior pituitary which results in thyroidal stimulation pro longed exposure to cold however results in a stressful situation and is accompanied by an increase secretion of adrenocorticotrophic hormone (ACTH)[123] which depresses thyroid function

As we have already seen[365] exposure of rats to an elevated temperature *reduces the requirement for thyroid hormone* Dempsey and Searles[366] have also demonstrated by histological methods a decrease of thyroidal activity in rats exposed to heat and Hurst and Turner[776] have shown that the rate of thyroid hormone secretion in mice maintained at an elevated temperature is depressed This is presumably due to a depression of TSH secretion in these animals Fontaine and Lachiver[510], Leloup[948] and others have produced evidence to support this view Further Knigge and Bierman[867] have found that in the hamster administration of the blocking agent reserpine or lesions in the median eminence affect the response of the thyroid to cold in hypophysectomized animals transplants of pituitary to the cheek pouch restored thyroidal ^{131}I uptake and release but did not restore the acceleration of release induced by cold The authors therefore conclude that the central nervous system is responsible for triggering the response of the thyroid to increased TSH secreted as a result of cold stimulation The mediation of the pituitary in the effect of temperature on the thyroid thus appears to be established in most species Tixier Vidal however[1622] has observed marked histological signs of cold stimulation in chick embryo thyroids although the chick embryo pituitary does not secrete TSH

(e) *Effect of the Adrenal Gland and Stress on Thyroid Function*

(i) *Adrenaline*—Adrenaline has been shown to produce thyroid hyper plasia and hypertrophy in rabbits and rats[592 1 05 1502] but not in hypo physectomized rats[1688] ^{131}I uptake is decreased by adrenaline in intact rats [03 1094] and rabbits[236] but increased in adrenalectomized animals

goitre by thyroxine has been shown by Dempsey and Astwood[365] to be quantitatively related to the dose of thyroxine This finding forms the basis of one of the simplest methods of assay for thyroid like potency and is extensively used today (see Appendix) Sellers and Schonbaum[1443] have observed an enhancement of the goitrogenic action of propyl thiouracil by small doses of thyroxine given over a long period The mechanism of this action is not known

The mode of action of the antithyroid drugs is not yet fully understood It has been suggested[12] that they act through the sulphydryl group by keeping iodine in the thyroid in the reduced state (iodide) thereby pre venting iodination of tyrosine There are two major objections to this simple explanation (i) If thiouracil and related compounds merely act by maintaining iodine in the reduced state their action should be overcome by administration of sufficiently large amounts of iodide (ii) This mechanism does not account for the goitrogenic action of the sulphona mides or resorcinol It is now generally considered that these drugs act by inhibiting the oxidizing enzymes involved in organic binding of iodine in the thyroid Evidence in support of this is found in the following reports Schachner *et al*[1416] showed the cytochrome oxidase inhibitors cyanide azide sulphide and carbon monoxide all prevented the formation of diiodotyrosine and thyroxine from ^{131}I in sheep thyroid slices Keston[852] found that the organic binding of ^{131}I by unpasteurized milk proteins in the presence of xanthine was inhibited by thiourea this finding was con firmed by Rosenberg[1380] (see [70 74]) Fraser and his colleagues[1219 527] have shown that many antithyroid drugs inhibit the activity of a partially puri fied xanthine oxidase and thereby prevent iodination of casein *in vitro* they suggested that compounds such as thiouracil inhibit the enzyme directly while resorcinol prevents iodination of the protein by removing the free iodine Astwood[70] has reviewed the mode of action of antithyroid drugs and concludes that inhibition of peroxidase activity in the thyroid is the most likely mechanism The goitrogenic action of large doses of iodide is described in Chapter 9

(d) Effect of Cold on Thyroid Function

The effect of temperature on thyroid function has been recognized for many years Seidell and Fenger[1433] found that the thyroid glands of sheep oxen and pigs in North America contained three times as much iodine during June to November as during December to May (see also [1506]) Riddle[1303] showed that the pigeon s thyroid enlarged and became more active during the winter months Ring[1308] found that cold stimulated the thyroid in rats and caused a rise in the BMR Histological evidence of thyroidal stimulation has been observed after exposure of rats and other animals to cold[1531 374 744]

thyroid function[1095] but its effect is feeble compared with that of cortisone[1072]

(ii) *Effects of other stresses*—Conditions of stress produced by starvation[1537 1874 2311 1094] radiation[1476 1241 1], burns[1695], tourniquet shock[850] restraint[126], formalin injection[1199] and other surgical and emotional stresses[1874 237 1094] have been shown to depress thyroid function in animals Gerwing *et al* [578] have demonstrated species differences in the response of the thyroid to injections of bacterial exotoxins which produced a depression of thyroid hormone secretion rate in the rat mouse and rabbit and an increased secretion rate in the guinea pig and rhesus monkey Gerwing[577] has further shown that in the rat continued stress will eventually result in hyperactivity of the thyroid Money[1094] has discussed the mechanism of action of different stresses on thyroid function and concludes that the effect is an indirect one resulting from adrenal stimulation and a consequent enhanced renal secretion of iodide

It is impossible in this brief survey on the effect of various stresses on thyroid function to mention individually all the workers who have contributed to this subject For more detailed reviews the reader is referred to articles by Money[1094] and Harris[705 706]

(IV) The Iodide Concentrating Mechanism of the Thyroid

As we have already seen the first stage in thyroid hormone biosynthesis is the concentration or trapping of iodide by the thyroid from the circulation The study of this mechanism has been facilitated in the past 18 years by the availability of radioactive isotopes of iodine whereby it has been possible to give tracer doses of iodide and investigate its passage into the thyroid under physiological conditions The work of Leblond and Sue[9 8] demonstrated the stimulation by the pituitary of iodide uptake by thyroid the passage of ^{128}I into rats thyroids could be greatly enhanced by administration of TSH and was in fact increased five fold after injection of 100 Junkmann Schoeller units Stanley and Astwood[15 9] studied ^{131}I turnover in the thyroid glands of normal humans during stimulation with thyrotrophin and found that it reached a peak 24–48 hr after a single injection the ^{131}I uptake did not return to normal levels for 4 or 5 days Morton *et al* [1119] demonstrated increased thyroxine synthesis in the thyroids of guinea pigs after administration of TSH Conversely hypophysectomy has been shown to depress concentration of iodide by the thyroid Morton *et al* [1118] showed that the uptake of ^{131}I by the thyroid of rats fell to only a small fraction of its normal value after hypophysectomy

Since the discovery of the antithyroid drugs of the thiocarbamide type which inhibit iodine binding in the thyroid it has been possible to study

[1501, 1094]. Reiss *et al.*[1, 93] found that adrenaline increased thyroidal ^{131}I uptake in normal humans but the response was absent in a patient with panhypopituitarism. Soderberg[1497] showed that small doses (2 μg) of adrenaline stimulated secretion of ^{131}I from the rabbit's thyroid but 4 μg was without effect. The mechanism of the adrenaline effect has been discussed by several authors (see references above; also [574, 1094]). It has been suggested (1) that adrenaline stimulates TSH secretion and so depresses thyroid ^{131}I uptake; (2) that it increases the peripheral utilization of thyroxine; (3) that it increases the level of blood ACTH in normal animals. ACTH has no effect on thyroid function in hypophysectomized animals but will inhibit the effect of TSH when given concurrently; adrenaline might therefore act indirectly on the pituitary via the adrenal cortex.

(ii) *Adrenocorticotrophic hormone*—Administration of ACTH has been found generally to depress thyroidal ^{131}I uptake in intact rats[1095, 1027, 1500, 22] but to have no effect on thyroid hormone secretion[1208, 2, 136]. Brown-Grant[30] however has found that ACTH depresses thyroid hormone release in normal rats but not in adrenalectomized animals. In the human, ACTH has been found to depress thyroid function[737, 1513] and to lower the level of thyroid hormone in blood[687]. It has been suggested[1094] that the raised renal excretion of ^{131}I that follows administration of ACTH [563, 886] may account for the depressed ^{131}I uptake.

(iii) *Adrenocortical steroids*—Cortisone has been stated to be without effect on thyroidal ^{131}I accumulation in the intact, hypophysectomized, TSH and propylthiouracil treated rat[661]; on the thyroid : serum iodide ratio in hypophysectomized or TSH treated rats[786] or on thyroid weight and histology in rats and dogs[1174]. Cortisone was found to have no effect on the peripheral utilization or rate of metabolism of exogenous thyroxine in treated cases of myxoedema[790, 436] or on the stimulation by exogenous TSH of thyroidal iodine uptake or hormone secretion in cases of panhypopituitarism or non-toxic goitre treated with thyroid hormone[792]. Many workers have, however, found that cortisone depressed ^{131}I uptake in rat and human thyroid glands[1095, 737, 1208, 529, 2, 1513, 155, 1094] (also see references relating to ACTH). The inhibitory action of cortisone on thyroid activity in mice was found to be greatly increased by simultaneous administration of adrenaline[862]. Cortisone also depressed thyroid hormone secretion in the rat and rabbit[1135, 238, 228, 32]. It seems likely that the discrepancies reported have resulted from the different doses of cortisone used.

Cortisone administration results in increased renal secretion of iodide in rats and man[886, 529, 155, 786]; for this reason Money[1094] suggests that the principal action of cortisone on the thyroid is a reduction of the amount of iodine available to the gland.

Deoxycorticosterone acetate (DOCA) has also been shown to depress

thyroid function[1095] but its effect is feeble compared with that of cortisone[1072]

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the view which is shared by VanderLaan[1669], that the collection of iodide is the rate limiting factor in thyroid hormone synthesis Ingbar and Freinkel[789] have described a method for the separation of iodide from organic iodine in rat thyroid by low temperature dialysis in human serum and have shown that there are concentration gradients of iodide in the thyroid even when iodine binding is unchecked they conclude that organification of iodine proceeds at a finite rate and may be the rate limiting factor in hormone synthesis

Halmi[660 664] has re evaluated the evidence relating to the control of the iodide concentrating mechanism by the thyroid hormone and finds that at present there is insufficient evidence to support the hypothesis that the thyroid gland is a target organ of its own hormone

Attempts have been made to learn something of this mechanism from experiments *in vitro* Wyngaarden *et al*[1755] studied iodide accumulation in homogenized thyroid tissue from rats given thiouracil in the drinking water or propylthiouracil in the diet The homogenates were consistently shown to bind iodide (^{131}I) across a Cellophane membrane but binding could be inhibited by thiocyanate and by propylthiouracil The bound iodide could not, however be discharged from the homogenate by thio cyanate it appears that the thiouracil or propylthiouracil present in the glands before the experiment became so diluted in the dialysis bags that some organic binding occurred Slingerland[1482] has also studied factors that influence iodide accumulation by the thyroid Sheep thyroid slices were incubated with ^{131}I under different conditions in the presence of propylthiouracil it was shown that iodide concentration was depressed in the absence of oxygen (cf [1027]) and in the presence of sulfhydryl inhibitors Neither these studies nor those described above have revealed the nature of the iodide concentrating mechanism of the thyroid gland

Other elements of the periodic group VII are also concentrated by the thyroid gland Perlman *et al*[1 04] showed that in rats and guinea pigs given the radioisotope of bromine ^{82}Br the thyroid gland concentrated bromine more than did other tissues (liver kidney brain pituitary and adrenals) the uptake was stimulated in guinea pigs by TSH Yagi *et al*[1758] have also studied the uptake of ^{82}Br by rat thyroid glands and have confirmed the finding of Perlman *et al*[1 04] The thyroidal uptake *of* ^{8} *Br was consider* ably less than the uptake of ^{131}I and no organic binding of bromine was demonstrable Manganese tellurium and rhenium are among the elements of this group that are concentrated by the thyroid[132 1453] The radioactive element 85 astatine (^{211}At) is also selectively concentrated by the thyroid gland but there is no evidence of its incorporation into organic com pounds[669 670 1699 968] It appears that the iodide concentrating mecha nism of the thyroid is not specific for iodine but that the oxidizing enzymes will only permit organification of this element

the iodide concentrating mechanism independently of hormone synthesis Astwood and Bissell[71] showed that prolonged administration of thiouracil to rats resulted in a fall in thyroidal iodine content to low levels Astwood[67] found however that thiouracil treated rats could still concentrate iodine when injected with potassium iodide VanderLaan and Bissell[1666] and VanderLaan and VanderLaan[1670] have shown that under the influence of propylthiouracil the thyroid gland of the rat is able to concentrate large amounts of iodide so that the thyroid serum iodide ratio may rise to a value of 250 1 This concentration of iodide is inhibited by thiocyanate and thiocyanate will cause the discharge of iodide already accumulated in the gland This work clearly demonstrates that the two types of anti thyroid drugs act on different stages of iodine metabolism

The response of the thyroid gland to the thiouracils depends as we have already noted on the integrity of the pituitary gland Astwood and Bissell[71] found that in hypophysectomized rats the thyroid gland could not reaccumulate iodide under the stimulus of thiouracil treatment Greer[619] VanderLaan and Greer[1668] and Randall and Albert[1266] also found that the thyroid glands of rats were dependent on the pituitary for the concentration of iodide from blood Halmi and co workers have shown[663] that hypophysectomy depresses the thyroid serum iodide concentration (T/S) to about one quarter of its normal value in rats prolonged administration of TSH in these animals restored the T/S ratio to normal or somewhat elevated levels Simultaneous administration of TSH and propylthiouracil caused a very great increase in the T/S values Halmi and Spirtos[662] showed that propylthiouracil administration to hypo physectomized rats failed to cause an increase in thyroid cell height TSH administration caused a significant increase in cell height and the effect of TSH was enhanced by simultaneous administration of propylthiouracil The authors concluded that their evidence lends support to the hypothesis put forward by Rawson and Money[1174] namely that propylthiouracil produces the characteristic hyperplastic changes in the thyroid by stimu lating TSH secretion from the pituitary and by an augmentation of its action on the thyroid

Wollman and Scow[1750] have studied the effects of hypophysectomy on thyroidal iodide trapping in the mouse After injection of a single dose of propylthiouracil the T/S ratio was 250 in normal mice and fell to a value of about 70 after hypophysectomy Hypophysectomy depressed thyroidal ^{131}I uptake in the eel[512] Lipner *et al* [966] have shown that transplants of thyroid lobes of mice to subcutaneous sites still possess the ability to concentrate iodide and show hyperplastic changes after propylthiouracil administration

Berson and Yalow[157] have studied the iodide concentrating mechanism of the thyroid in clinically euthyroid and hyperthyroid patients they hold

hormone biosynthesis further hypophysectomy inhibits directly the conversion of monoiodotyrosine to diiodotyrosine as well as other processes of thyroid hormone metabolism These effects of hypophysectomy on thyroid function are shown in Fig 3

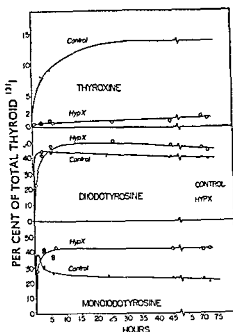


Fig 3

Distribution of ^{131}I in iodinated amino acids present in hydrolysates of thyroid glands from normal and hypophysectomized rats in function of time after the administration of ^{131}I Hypx = hypophysectomized (From Taurog Tong and Chaikoff[1600])

(VI) Iodine Metabolism in the Developing Thyroid Gland

Although a few studies on the function and incorporation of stable iodine into the embryonic thyroid have been reported[475, 749, 1553] it was not until ^{131}I became available that thyroid hormone synthesis in prenatal life could be easily investigated Gorbman and Evans[598] showed that incorporation of ^{131}I occurs in the thyroids of tadpoles of the frog *Hyla regilla* very soon after the formation of follicles The ability of the foetal thyroid to store iodine begins between the eighteenth and nineteenth day of gestation in the rat[599] on the fifty second day in sheep[119] and between the twelfth and fourteenth week in the human[278, 1509, 985] Chaikoff and co workers[1745, 874] have found that in the foetal calf there is a gradual development of the thyroid accompanied by a steady increase in organic

(V) Organic Binding of Iodine in the Thyroid as Influenced by the Anterior Pituitary

The anterior pituitary secretion of TSH has been shown to have a profound effect on the thyroid gland *in vitro* The first attempt to demonstrate a direct action of anterior pituitary extracts on isolated thyroid tissue[367] failed but in 1933 Litel *et al* [425] found that typical morphological changes occurred in thyroid slices when they were incubated with TSH Paal[1188], Anderson and Alt[46] and Canzanelli and Rapport [58] have all reported increases in the oxygen consumption of thyroid slices in the presence of TSH Morton and Schwartz[1120] have found that TSH enhances the incorporation of ^{32}P into the phospholipids of thyroid slices

Bakke and Lawrence[86] and Roche *et al* [1369] have shown that the presence of anterior pituitary extract markedly stimulates iodine uptake and biosynthesis of the iodotyrosines and iodothyronines in slices and tissue cultures of rat thyroid Lacy *et al* [897] and Oppenheimer *et al* [1175] however, have been unable to demonstrate any effect of TSH on organic binding of ^{131}I by thyroid tissue *in vitro* Botkin *et al* [92] showed a depression of ^{131}I uptake in whole rat thyroids incubated with TSH isolated rat thyroids from animals pretreated with TSH also showed decreased ability to concentrate and bind ^{131}I compared with glands from untreated animals[451]

The effects of hypophysectomy on ^{131}I metabolism in the thyroid have also been studied Morton *et al* [1116] showed that the uptake of ^{131}I in the thyroids of hypophysectomized rats was markedly depressed further, thyroxine synthesis appeared to be inhibited to a greater extent than diiodotyrosine synthesis Albert and Lorenz[19] did not observe preferential inhibition of thyroxine synthesis after hypophysectomy Roche *et al* [1335] showed that hypophysectomy resulted in a high ratio of labelled monoiodotyrosine to diiodotyrosine in the rat thyroid and relatively less thyroxine than in the glands of the control animals they concluded that thyrotrophin has no specific action on thyroxine biosynthesis Cetini *et al* [266] were unable to detect any thyroxine 24 and 48 hr after ^{131}I injection in the thyroids of rats that had been hypophysectomized for 17 days

Taurog *et al* [1589] have extended the studies of Morton *et al* [1116] and have found that in the absence of the pituitary the accumulation of iodide the formation of the iodotyrosines and thyroxine and the rate of secretion of thyroxine into the blood are all depressed Recently these authors [1590, 1591] have shown that the total ^{131}I uptake and the ratio of diiodotyrosine ^{131}I to monoiodotyrosine ^{131}I is markedly depressed in hypophysectomized rats injected with ^{131}I the thyroxine ^{131}I was also depressed Single or repeated doses of TSH reversed the effects of hypophysectomy The authors present evidence that TSH acts independently on the iodide concentrating mechanism of the thyroid and on the different stages of thyroid

development of the foetal thyroid and the appearance of iodine, diiodotyrosine and thyroxine at different embryonic ages the stage at which it responds to thyrotrophic hormone in various species is also reviewed

Jost[821] has studied the action of propylthiouracil on foetal thyroid function in the rat in the normal foetus administration of propylthiouracil to the mother produces marked hypertrophy of the foetal thyroid, this does not occur in the decapitated (hypophysectomized) foetus It appears that in the rat the foetal thyroid is not controlled by the foetal pituitary gland

(VII) RATE OF SECRETION OF THE THYROID HORMONE INTO THE CIRCULATION

Little direct evidence for the sequence of events occurring during thyroid hormone biosynthesis has been described it has rather been inferred from the effects of hypophysectomy on intrathyroidal iodine metabolism Early estimates of the rate of thyroid hormone secretion from the human thyroid were calculated from the amount of thyroxine required to restore the myxoedematous subject to a clinically euthyroid state[1236, 199, 1601] It was found that the daily requirement of myxoedematous patients was 0 3–0 35 mg DL thyroxine Means[1084] estimated the daily requirement of U S P thyroid in hypothyroid humans and found it to be about 100 mg Greer[621] finds a somewhat higher requirement Tubiana[1645] has found the daily turnover of thyroxine iodine in man to be 250 µg Hamolsky *et al*[679] gave this figure as 119 µg in euthyroid subjects

Wolff[1742] has shown that the rate of secretion of thyroid hormone in rats is markedly influenced by the pituitary The rate of secretion was measured by external counting of the thyroid after the administration of ^{131}I with and without propylthiouracil treatment He found that the biological half life of the ^{131}I labelled hormone in the thyroids of the control animals was 3 3 days hypophysectomy or administration of DL thyroxine (15 µg) increased the half life to 24 and 26 days Injection of thyrotrophin stimulated the release of hormone from the gland and the biological half life fell to 8–10 hr Goldsmith *et al*[596] have used the measurement of the biological decay rate of hormonal ^{131}I to study secretion in thyrotoxic patients 2 mercapto 1 methylimidazole (methimazole) was found to increase the rate of secretion in three of the six subjects studied and the simultaneous administration of TSH caused a further increase in the rate of secretion in all six subjects

Solomon[1504] has studied factors that influence thyroid hormone secretion in euthyroid and hyperthyroid subjects During methimazole treatment the secretion rate was faster in hyperthyroid than in euthyroid patients (7 025% per day compared with 1 911% per day) Administration of

iodine content thyroxine appears between the fifty third and seventieth day of gestation Gorbman *et al*[600] injected ^{131}I into pregnant cows that were near term and killed the animals 24 hr later the foetal thyroids contained more than twice as much ^{131}I as the maternal thyroids and also had a much higher thyroxine ^{131}I content The authors concluded that the disparity in the TSH content of the maternal and foetal circulations was responsible Similar studies have been made in sheep[118]

The foetal rabbit thyroid begins to concentrate ^{131}I between the 15th *and 16th days of gestation and to bind iodine about 2 days later*[1897] at no stage of iodine binding were the precursors of thyroxine found without any thyroxine itself Geloso[561] administered ^{131}I to pregnant rats and found labelled iodotyrosines and iodothyronines in the foetal thyroids after 22 days of gestation Jost *et al*[8 5] and Geloso[562] have hypophysectomized rabbit foetuses by decapitation *in utero* and have shown that this inhibits the uptake and release of ^{131}I by the foetal thyroid neither hypophysectomy nor thyroidectomy however affected the growth of the foetus Geloso (see also [823]) finds that 3 hr after injection of ^{131}I into the pregnant rat on the 21st day of pregnancy the foetal thyroid contains more thyroxine and triiodothyronine than the maternal thyroid

Hansborough and Khan[685] have shown that ^{131}I is incorporated into the chick embryo on the 11th day at the same time as discrete thyroid follicles first appear Wollman and Zwilling[1751] found that ^{131}I was con centrated by the chick embryo on the 9th day and most of it was protein bound Trunnell and Brayer[1643] have found that administration of TSH increases the amount of ^{131}I collected in the chick embryo thyroid but does not alter the time at which it begins to collect iodine Meyniel *et al*[1078] have demonstrated the presence of thyroxine in the body of the chick embryo from the 10th day of embryonic development Trunnell and Wade[1644] have demonstrated that iodide concentration and binding occur at successive time intervals in the chick embryo thyroid At day 7 5, only iodide could be detected after injection of ^{131}I at day 8 5 monoiodotyrosine appeared followed by diiodotyrosine on day 9 25 and thyroxine on day 9 75 It appears that these thyroid glands elaborate the mechanisms for *concentrating iodide and for synthesizing the iodotyrosines and thyroxine* in definite stages and it is of particular interest that there is a period when monoiodotyrosine but not diiodotyrosine can be formed Pitt Rivers *et al*[12 7] suggest that the formation of monoiodotyrosine is a primitive process which can occur in thyroid homogenates[1588] and in the mammary gland[234] it is also the principal iodinated constituent of thyroid protein in certain pathological conditions[1577 1229 312] The formation of diiodo tyrosine and the thyroid hormones require special enzymes which are formed later in foetal development and which are destroyed or absent in disrupted and pathological thyroid tissue Willier[1728] has discussed the

et al[113] These authors found that the thyroidectomized rat required 12 μg DL thyroxine daily to restore the BMR to normal values

Riggs[1305] considers that estimates of thyroid secretion in man based on replacement therapy gives values which are too high, while estimates based on uptake of ^{131}I and excretion of stable iodine are too low He proposes that the normal secretion rate in man is about 70 μg of thyroxine iodine Hurst and Turner[777] have also discussed the relative merits of the goitre prevention technique and measurement of ^{131}I labelled hormone for the estimation of thyroid secretion rates

iodide depressed the rate of release in a patient with Graves' disease (see also [6 6 595]) Administration of TSH to euthyroid subjects increased the rate of hormone release

Berson and Yalow[156] have calculated the rate of thyroid hormone secretion from the kinetics of radioactive organic iodine between the thyroidal and extrathyroidal pools reincorporation of ^{131}I was blocked by methimazole administration The secretion rates in euthyroid subjects ranged from 77 to 125 μg per day, but rose to 450–925 μg per day in untreated cases of thyrotoxicosis (see also [154]) Nodine *et al* [1162] have found that the normal human thyroid contains a store of 5 26 mg of iodine and secretes 56 μg of iodine daily

Randall *et al* [1267] have shown that hypophysectomy depresses thyroid hormone secretion to 13% of the normal secretion rate in rats for several days after the operation Perry[1207] Albert and Tenney[21] and Reineke and Singh[1285] have also found suppression of thyroid hormone release by exogenous thyroxine the latter authors calculated that the secretion rate of thyroid hormone in rats was 2 56 μg per 100 g body weight per day in animals given thiouracil the secretion rate was 10% higher

Thyroid secretion rates have been measured in sheep[723] pigs[1506] rabbits[233] and in several strains of hybrid mice[44] in these mice the secretion rates were significantly higher in some strains than in others while the rates in the hybrids were intermediate between those of the parent strains The authors concluded that this aspect of thyroid function is under genetic control

In 1943 Dempsey and Astwood[365] devised a new method for calculating the daily secretion rate of the thyroid hormone in the rat The animals were maintained on thiouracil and the dose of L thyroxine required to prevent the development of goitres was determined at normal low and elevated temperatures At 25 C the daily secretion rate was found to be 5 2 μg thyroxine this rose to 9 5 μg when the rats were kept at 1 C and fell to 1 7 μg when the temperature was raised to 35 C This method for determining the daily rate of thyroid hormone secretion has often been employed By this means Biellier and Turner[166] and Hoffmann[745] have found that the duck secretes 18 μg thyroxine per day during the first week of goitrogen administration The daily secretion rate in terms of DL thyroxine has also been determined in white leghorn chicks[1166] and in growing turkey poults[167] Maqsood[10 3] used this method for estimating thyroid hormone secretion in the rabbit and found that it decreased with advancing age when calculated per 100 g body weight The secretion rate for young rats was higher than that for old rats[604]

The observation of Meyer and Wertz[1075] that thyroidectomized rats are more sensitive to the thyroid hormone than are intact animals forms the basis for the method of determining thyroid secretion rate of Barker

goitre[1521, 1250] The presence of iodoproteins or peptides of thyroidal origin in the blood in a wide variety of pathological conditions has recently been described, thyroglobulin has been detected in the blood of patients with lymphadenoid goitre or Hashimoto's disease[1187, 1374, 1737, 387, 603, 818] in animals with chronic thyroiditis[1737] and after surgical or radiation thyroidectomy[1321, 16, 8, 1314, 1315] Another circulating iodoprotein that has been well characterized is Compound X which is found in patients with carcinoma of the thyroid it has a molecular weight about one tenth that of thyroglobulin after hydrolysis the only iodinated compound found in this protein is monoiodotyrosine[1324, 1577, 1575] Several reviews relating to normal and abnormal iodinated compounds in the blood have recently appeared[107, 1226, 1575]

(II) The Binding of Thyroid Hormones to Serum Proteins

Thyroxine Binding Protein

Trevorrow[1632] observed that thyroxine in the blood was not dialysable and could be coprecipitated with plasma proteins it was later shown that all the thyroxine which was thus precipitated was easily extractable by *n* butanol[1582] It is apparent from these facts that the circulating thyroid hormone is bound in some way to one or more of the plasma proteins so firmly that the link cannot be broken by dialysis or by reagents such as trichloroacetic acid and zinc hydroxide it can however be broken by extraction with large amounts of ethanol or butanol[942, 173] solvents in which thyroxine is readily soluble These properties of thyroxine have found widespread clinical application in the determination of protein bound iodine (PBI) serum protein iodide (SPI) and butanol extract able iodine (BEI) these levels represent a balance between thyroidal hormone secretion rate and its peripheral disappearance rate[1213]

Early attempts to discover the nature of the binding of thyroxine and other iodinated substances to serum proteins by salting out procedures showed that iodine was present in most protein fractions the largest amount was found in the albumin and α globulin fractions[1406] Taurog and Chaikoff[1583] also reported that the largest amount of blood iodine was present in albumin but the highest concentration was in the α globulins Somewhat similar observations were made in experiments with moving-boundary electrophoretic separation of serum proteins[1463] Later Gordon *et al*[601] used zone electrophoresis on paper to investigate the behaviour of ^{131}I labelled thyroid hormone at physiological levels in blood they found that the endogenous labelled hormone in the plasma of hyper thyroid patients treated with ^{131}I or trace amounts of synthetic labelled thyroxine added to normal plasma behaved identically and migrated on electropherograms at pH 8 6 to the α globulin zone a small amount of radioactivity accompanied the albumin fraction These results are illus

CHAPTER 4

TRANSPORT OF THE THYROID HORMONE

(I) The Circulating Thyroid Hormone

The nature of circulating thyroid hormone was debated for many years after the discovery of thyroxine in the thyroid gland At first it was thought that the hormone secreted into the blood might be a peptide or polypeptide containing both thyroxine and diiodotyrosine[69 1405 1407] This belief *arose mainly from the facts (1) that the thyroid hormone in* plasma was precipitated by protein precipitants (2) that thyroxine was not found to have as high a potency as whole thyroid substance with the same thyroxine content (this can now be explained by the presence of the more potent triiodothyronine in thyroid tissue) Further, early work had *indicated that blood iodine was not entirely extractable with alcohol*[753 992 384] The first experimental evidence in favour of thyroxine itself being the circulating hormone came with the publication of Trevorrow in 1939[163] who showed that thyroid hormone in the blood behaved in the same way towards protein precipitants dialysis and ethanol extraction as *did added thyroxine Shortly afterwards Lerman*[950] *showed by immuno* logical methods that thyroglobulin is not normally present in the circulation By 1944 Harington[693] in a reconsideration of the nature of circulating thyroid hormone concluded that it was indeed thyroxine it was not until four years later that Taurog and Chaikoff[158] demonstrated unequivocally *that the circulating hormone labelled with* ^{131}*I and added labelled thyro* xine showed identical behaviour towards protein precipitants and organic solvents this was confirmed by Leblond and Gross[924] and additional evidence in favour of thyroxine as the circulating hormone was presented in the chromatographic studies of Laidlaw[898] Since then it has been repeatedly shown that the principal thyroid hormone in the blood of most *animals is thyroxine it is as we have already seen usually accompanied* by small amounts of triiodothyronine (see Chapter 2) Many workers have however failed to detect triiodothyronine in plasma and from quantitative considerations thyroxine represents the major thyroid secretion in the circulation

Other iodinated compounds synthesized by the thyroid (see Chapter 2) have also been described in the blood the presence of 3 3 diiodothyronine and 3 3 5 -triiodothyronine[1359 1356] in rat blood has yet to be confirmed The secretion of iodotyrosines into the blood is only found in abnormal circumstances and perhaps in certain subjects with congenital

✓goitre[1521 1250] The presence of iodoproteins or peptides of thyroidal origin in the blood in a wide variety of pathological conditions has recently been described, thyroglobulin has been detected in the blood of patients with lymphadenoid goitre or Hashimoto s disease[1187 1374 1737 387 603 818] in animals with chronic thyroiditus[1737] and after surgical or radiation thyroidectomy[13 1 1628 1314 1315] Another circulating iodoprotein that has been well characterized is Compound X which is found in patients with carcinoma of the thyroid it has a molecular weight about one tenth that of thyroglobulin after hydrolysis the only iodinated compound found in this protein is monoiodotyrosine[1324 1577 1575] Several reviews relating to normal and abnormal iodinated compounds in the blood have recently appeared[107 1226 1575]

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trated in Fig 4 These findings were soon confirmed, but it was found that thyroxine binding occurred on a protein fraction that migrates between α_1- and α_2 globulins[1317 1732 910 762] This α globulin fraction is now commonly referred to as thyroxine binding protein or TBP This nomenclature is one of convenience since thyroxine is not the only iodinated compound that this protein will bind, nor has it been shown to consist of a single component

It must be emphasized that the binding of thyroxine to serum pro teins in no way *resembles the peptide linkage by which thyroxine is bound* in thyroglobulin

(a) *Attempts to Isolate and Characterize Thyroxine binding Protein*

Purification of TBP is essential in order to determine its physical and chemical properties and to interpret its physiological importance In the earlier work based on the measurement of iodine nitrogen ratios of protein fractions obtained by precipitation by ethanol[1407] and by metal ions (Cohn method [95]) the major iodine containing fractions were found to be the Fractions IV (rich in α globulins) and V (albumin) no method was however available for labelling the specific TBP at that time and it was impossible to say whether these fractions represented a concentration of the binding protein or an artificial concentration of iodine brought about by Cohn s procedures Later when the thyroxine binding protein was studied in serum containing endogenously or exogenously ^{131}I labelled thyroid hormone Robbins and colleagues[1209 1316] were able to show that in its electrophoretic properties in buffers of pH 8 6 or 4 5 and sedimen tation in the ultracentrifuge TBP as represented by the major radioactive fraction resembled the α_2 glycoproteins of Schmid[1423 1424] or the muco proteins (M 2 glycoproteins) of Mehl *et al*[1066] (For TBP, moving boundary electrophoresis gives a value for the mobility of $\mu \cong -4\ 7 \times 10^{-5}$ cm volt $^{-1}$ sec^{-1} at pH 8 6 and $\mu \cong -1\ 7 \times 10^{-5}$ cm^2 volt^{-1} sec^{-1} at pH 4 5 the sedimentation constant $S_{20\ w} = 3\ 3$ corresponding to a molecular weight of about 50 000) Schmid[1423] used the determination of iodine nitrogen ratio to identify TBP and concluded that the major part of serum iodine was present in the mixture of α globulins in Fraction VI obtained by Cohn s method No 6 However when the thyroxine binding affinity of different Cohn fractions were studied by the addition of labelled thyro xine Freinkel *et al*[531] obtained different results, they found that the Cohn subfractions IV 6 and IV-9 derived from IV 4 exhibited the highest TBP content This localization of thyroxine binding activity has enabled Ingbar and his colleagues to use Fraction IV 6 as the starting material for purification of TBP[788] These workers have been successful in enriching TBP by the use of Dowex 1 ion exchange resin but it has not yet been shown that TBP is a single protein The disadvantages of procedures



Fig. 4

Protein binding of endogenously and exogenously labelled thyroxine by human serum proteins

(a) Distribution of serum proteins after electrophoresis on paper. Serum from a euthyroid subject 5 days after 100 mc ^{131}I for treatment of thyroid carcinoma.

(b) Autoradiograph of strip (a) showing almost all the radioactivity of endogenously labelled thyroxine in the α-globulin zone with a trace in the albumin region.

(c) Paper electrophoretic distribution of serum proteins from an untreated euthyroid subject to which synthetic ^{131}I labelled thyroxine was added before electrophoresis.

(d) Autoradiograph of strip (c) showing the similarity of binding of endogenously and exogenously labelled thyroxine.

(e) Autoradiograph of electropherogram of ^{131}I labelled thyroxine alone.

(f) Electrophoretic pattern obtained in the Tiselius apparatus of the serum sample shown in (c).

(From Gordon, Gross, O'Connor and Pitt-Rivers)

L-THYROXINE IN NORMAL SERUM

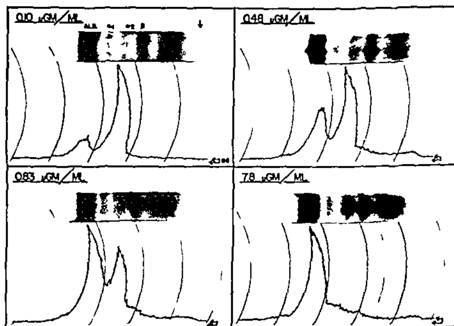


Fig 5

Displacement of ^{131}I labelled thyroxine from thyroxine binding protein (α_1–α_2 globulin fraction) to serum albumin by the addition of increasing amounts of thyroxine to human serum. The curves represent the distribution of thyroxine radioactivity in different serum protein fractions as shown by the accompanying strip showing the electrophoretic migration of serum proteins. The figures represent the final concentration of thyroxine added to the serum. (From Robbins and Rall[314].)

involving ethanol fractionation in the presence of bivalent cations such as zinc and the use of ion exchange resin are that the endogenous hormone present in the serum is dissociated from the protein to which it is bound and (in the case of ion exchange resin) *the binding potency is modified*

Thus we may conclude that TBP is represented by one or more of the α globulins present in serum in small amounts it has a molecular weight of about 50,000 and an isoelectric point below pH 4 5, it is likely that it is a glycoprotein It has been suggested that the carbohydrate in TBP is the part of protein responsible for binding there is at present no firm theoretical basis for such an assumption nor is there any supporting experimental evidence

(b) Binding Properties of Thyroxine binding Protein

Even though TBP has not been isolated it has been possible to gain some information about its properties because of the ease with which it can be labelled either in whole serum or in mixtures of proteins of unknown composition by the addition of minute amounts of radioactive thyroxine and related compounds

Of all the derivatives of thyroxine studied TBP has the highest binding affinity for thyroxine itself This finding results from studies in which increasing amounts of a thyroxine analogue are added to serum containing a trace amount of labelled thyroxine The relative binding capacity of TBP for the analogue can be estimated from the concentration of the analogue needed to produce a given amount of displacement of the labelled thyroxine from the TBP zone in paper electrophoresis Some of the results so obtained by Robbins and Rall[1319] and Larson and Albright[909] are presented in Table I An examination of this table leads us to the conclusion that all the major groups of the thyroxine molecule i e the four iodine atoms in the 3 5 3 5 positions the phenolic hydroxyl the alanine side chain and the diphenyl ether linkage contribute towards the maximal binding of this hormone Larson and Albright[909] have found that D thyroxine is less firmly bound to TBP than is L thyroxine Robbins and Rall[1319] however find little difference between TBP binding of L- and D thyroxine It is impossible to say exactly how the iodothyronine molecule is attached to the protein until purified TBP is available although various possibilities have been suggested[106 13 0] The techniques used so far have certain disadvantages and require confirmation by *other methods* For example if an analogue is bound to other serum proteins the resultant displacement of thyroxine will not represent the affinity of TBP for this particular substance in this connection 3 3 -diiodo DL thyronine has been shown to be almost entirely bound to serum albumin while TBP has only a slight affinity for it[13 0]

The capacity of TBP to bind thyroxine has been measured by raising

TABLE I

Relative affinity of thyroxine binding protein (TBP) for L thyroxine and its analogues

Compound	Relative affinity
L Thyroxine	100
D Thyroxine	28 80
Ortho thyroxine	5
Meta thyroxine	3
o Methylthyroxine	6
3 5 3 5 Tetraiodothyropropionic acid	3
Thyroxamine	7
3 5 Diiodo 3 5 dinitro DL thyronine	47
3 5 3 5 Tetranitro DL thyronine	1
3 5 Diiodo 3 5 dimethyl DL thyronine	2
3 5 3 Triiodo L thyronine	33
3 5 Diiodothyronine	0
3 5 Diiodotyrosine	Negligible*

* The binding of labelled 3 5 diiodotyrosine was measured directly

the level of endogenous hormone by increasing additions of synthetic thyroxine by this means the amount of endogenous hormone displaced from TBP can be estimated Good agreement has been obtained by many workers[24 1318 531 994] on the saturation of TBP with extraneous thyroxine *in serum from hypothyroid euthyroid and hyperthyroid subjects* In general TBP in euthyroid serum (which has a thyroxine concentration of about 10- M) is about one third saturated with respect to thyroxine When the concentration of thyroxine is raised above 3×10^{-7}M it is bound in increasing amounts to serum albumin The converse has also been shown to take place Dingledine *et al*[379] have shown that thyroxine bound to isolated albumin will be transferred to TBP if serum is added Although serum albumin has a lower binding affinity than TBP its higher concentration in serum makes it a secondary binding site which is more difficult to saturate Thus at a concentration of about 10^{-5}M in serum thyroxine is almost entirely bound to albumin This effect of spilling over of thyroxine from TBP to albumin is shown in Fig 5

TBP has also been shown to bind triiodothyronine *in vitro* this binding is weak and TBP can be more easily saturated with respect to triiodothyronine than thyroxine[460 3 9] Even albumin easily gets saturated with increasing concentrations of triiodothyronine and the spilling over continues on to other globulin fractions This relatively weaker binding of triiodothyronine is also demonstrated by the ready displacement of

bound triiodothyronine by added thyroxine and by the difficulty of displacing thyroxine by triiodothyronine

These studies not only give information on the binding capacity of TBP but demonstrate the existence of a simple equilibrium between free thyroxine and thyroxine bound to TBP or other binding proteins The time required to attain this equilibrium has not been calculated but it appears to be extremely short Lein[939, 940] using the technique of equilibrium dialysis has found that although bovine serum albumin has two types of binding sites for thyroxine (four and eight binding sites per protein molecule) only one type (eight binding sites per molecule) is effective at concentrations of thyroxine below $0\ 5 \times 10\ ^{4}$M Equationl derived for a single binding constant were said to fit the experimentas data Unfortunately Lein s work has so far been available to us only in abstract form and it is difficult to judge the accuracy of his values Also the pH of 9 75 which he used in his experiments might be the cause of significant error Recently Tata (unpublished) has found that an increase in pH will open up or activate many protein groups that increase the binding of thyroxine and may even cause a normally non binding protein to develop binding capacity thus γ globulin which has no significant binding activity at pH 7 4 exhibits a marked binding affinity for thyroxine at pH above 10 5 Robbins and Rall[1320] have however derived values for the association constant for TBP and thyroxine present in human serum they measured the displacement or binding capacity at different concentrations of thyroxine by paper electrophoretic analysis Since most of the serum thyroxine is bound to TBP and albumin Robbins and Rall[1320] have also used for human serum albumin the association constant obtained by Lein for bovine serum albumin It is not yet certain whether human and bovine serum albumin have similar binding properties for thyroxine They have further assumed that both human TBP and serum albumin have a single type of binding site and that protein protein and thyroxine thyroxine interactions are negligible In this way they arrived at a value of $7\ 9 \times 10^{9}$ for the association constant of human TBP Such a high figure is remarkable (but not surprising considering the low concentrations at which both TBP and thyroxine are present in blood) and indicates the very low amounts of free thyroxine that must be present in the blood It is impossible to measure directly the level of free thyroxine in serum but its value can be deduced from the association constant and level of total serum thyroxine Thus Robbins and Rall have arrived at a figure of $3–9 \times 10\ ^{11}$M as the concentration of free thyroxine in normal human serum this represents an average of about 0 06% of the total serum thyroxine However the accuracy of these figures can only be confirmed after the above assumptions have been proved correct from results obtained with different techniques and studies carried out with pure TBP

(*c*) *Comparative Aspects of Thyroxine binding in Serum*

So far only the thyroxine binding properties of human serum have been described in detail results of similar studies in other animals are beginning to appear Robbins and his collaborators (Apter Robbins and Rall cited in [1330]) have described briefly the electrophoretic distribution of radioactivity after the addition of ^{131}I labelled thyroxine to the serum of eight mammals and two reptiles more detailed accounts are also available for rat[1138] and sheep[47] serum Serum from the calf, sheep, pig goat mule and horse all exhibit thyroxine binding comparable to that in human serum in that most of the thyroxine is bound to proteins in the α globulin zone with a small amount of binding on the albumin fraction In the rat the specific thyroxine binding protein is difficult to distinguish electrophoretically from albumin[1330] but Myant[1138] has been able to demonstrate the existence of a TBP by variations of the buffers and pH of electrophoresis Dog serum shows three zones of thyroxine binding albumin α globulin and β globulin the largest fraction of added thyroxine (0 01 µg/ml) being bound to albumin Both the alligator and crocodile have two binding proteins (one being albumin) in the serum, but the presence of TBP is not clear in the crocodile

The binding of thyroxine and triiodothyronine to serum proteins in two species of birds has been recently studied in greater detail and the following observations have been made (Tata and Shellabarger, unpublished) the serum of both the fowl and duck is deficient in a thyroxine binding protein in the α globulin zone and binding occurs only in the albumin zone This has been substantiated by studying the binding by proteins after ethanol fractionation Further thyroxine binding by whole fowl serum is similar to that by isolated fowl albumin and is feeble, compared with that of mammalian serum a small amount of human serum or purified human TBP (Cohn fraction IV 6) added to a sample of fowl or duck serum containing ^{131}I labelled thyroxine very easily causes a transfer of thyroxine from fowl serum albumin to human serum TBP These studies have been made with the aid of electrophoresis and a new method described on page 58 The binding affinity and capacity of fowl serum albumin unlike that of human TBP does not vary significantly for thyroxine and triiodothyronine the significance of these findings will be discussed later

(*d*) *Conditions that Modify Thyroxine binding in Serum*

In most thyroid disorders in man little variation has been found either in qualitative or quantitative aspects of binding of thyroxine by whole serum or TBP It might be inferred that TBP in the serum of a hypothyroid subject is less saturated and in a hyperthyroid subject more saturated, than in the euthyroid subject These were essentially the

findings of Albright *et al* [24] and of Robbins and Rall[1320] though the latter authors believe that the concentration of TBP in serum is slightly elevated in hypothyroidism It has been suggested that there exists a qualitative difference in the thyroid *hormone plasma protein complex in hyper* thyroidism especially in subjects with diffuse toxic goitre[530 677] It is not known whether this reflects a difference in the properties of TBP since the methods used in these studies (a comparison of the disappearance rates in the rat and dog of thyroxine and triiodothyronine present in the plasma obtained from subjects with different thyroid disorders) can only give an idea of the extent of saturation of the binding protein

Interesting observations have been made on changes in thyroxine binding in certain physiological or pathological states i e pregnancy and nephrosis Heinemann *et al* [720] noticed an increase in serum protein bound iodine (7–13 μg/100 ml) at an early stage during pregnancy and the increased iodine was later shown to represent thyroxine[347 480] This high level of circulating thyroid hormone is not accompanied by an increase in basal metabolism[1408] It was suggested[347 1018] that there might be an alteration in the protein binding of thyroxine in pregnant women This was later shown to be the case Dowling *et al* [393 394] demonstrated that there was an increase in the thyroxine binding capacity of TBP in serum of pregnant women and that it was due to an absolute increase of TBP They also showed that patients with a diagnosis of threatened abortion failed to demonstrate a rise in TBP concentration similar findings have been reported by Robbins and Nelson[1313] Dowling *et al* [395] have also shown that it is possible to raise the level of serum TBP in normal men *and women by administration of diethylstilboestrol thus explains the* increased protein bound iodine reported earlier in normal subjects treated with oestrogen[437] However Feldman[469] has not been able to show any direct effect of oestrogen on thyroid hormone secretion in the rat

In nephrotic patients it is quite common to find symptoms of deficient thyroid function e g an elevated serum cholesterol a depressed BMR and low protein bound iodine but these patients do not react to thyroid hormone therapy as do true hypothyroid subjects[440 941 1211] and also exhibit a shortened half life of thyroid hormone[1270 340] The report of increased loss of hormonal iodine in the urine of these patients[1305] was followed by reports of the abnormal binding of thyroxine to protein[13 1 77] However Robbins *et al* [1323] have later shown that such an abnormal binding was only apparent owing to errors inherent in the experimental method, and conclude that there is no qualitative difference in TBP of normal or nephrotic subjects on the other hand they find that the thyroxine binding capacity of TBP in sera of these patients is considerably lower than in normal subjects In fact Freinkel *et al* [534] have detected TBP in the urine of patients with nephrosis These findings explain the

low circulating hormone levels and the large urinary losses of hormonal iodine in nephrosis

(III) THYROXINE BINDING BY OTHER SERUM PROTEINS EXTRAVASCULAR FLUIDS AND TISSUE PROTEINS

(a) *Other Serum Proteins*

There have been few reports concerning binding of endogenously labelled or added radioactive thyroxine or triiodothyronine to proteins other than the α globulin type TBP and albumin However the failure to detect binding on other proteins by the methods available so far (principally electrophoresis) does not necessarily exclude the existence of other thyroxine binding proteins It does in fact appear that the thyroxine binding property is manifested to a marked degree by a protein fraction with a greater electrophoretic mobility than that of serum albumin this has been termed component X V component or more commonly the Pre albumin of Esser and Heinzler[45] Human cerebrospinal fluid (CSF) has a relatively high concentration of this fast moving protein[351] and it was in CSF that thyroxine binding to pre albumin was first observed[38, 130] further pre albumin was found to be one of the principal carriers of thyroxine Robbins and Rall[130] found that as much as 35% of radioactivity of labelled thyroxine added in small amounts to concentrated CSF migrated with pre albumin Pre albumin has been isolated from human serum and purified and has been found to bind thyroxine more strongly than enriched TBP or any other protein fraction (Tata unpublished Ingbar unpublished also[199]) Only very little pre albumin is needed to displace thyroxine from TBP Tata (unpublished) has shown by two methods that pre albumin prepared by Schultze[1433] had thirty times the thyroxine binding affinity of albumin and six times that of TBP in Cohn fraction IV 6 At present pre albumin is the protein with the highest known affinity for thyroxine The physiological significance of the binding of thyroxine to pre albumin is not known There is some physicochemical difference between albumin and pre albumin but the latter has an exceptionally high tryptophan content[1433] Whether this is in any way connected with thyroxine binding remains to be discovered

The β and γ globulins have little or no thyroxine binding capacity However Robbins and Rall (personal communication) have found one patient with thyroid carcinoma *in whose serum most of the thyroxine* was bound to γ globulin even in the presence of TBP and albumin but it is worth noting that normal γ globulin and also many other proteins, bind thyroxine *in vitro* in increasing amounts as the pH of the medium is increased at pH 8 6 the most commonly employed for investigation of thyroxine binding in serum there is a slight but significant binding by γ globulin

(b) Extravascular Fluids

Thyroxine binding has also been observed in many extravascular fluids Alpers and Rall[38] showed that endogenous and exogenous thyroxine were protein bound in human cerebrospinal fluid to a protein fraction similar to serum TBP in electrophoretic mobility at pH 8 6 Since then TBP has been demonstrated to be the principal and albumin the secondary thyroxine binding protein in the following fluids thoracic duct lymph femoral lymph ascites fluid and joint fluid[534] A comparison of thyroxine binding capacity of the TBP of hydrothorax chyle CSF and serum revealed that in the pleural fluid the concentration of TBP and thyroxine was nearly the same as in serum whereas in the spinal fluid it was about 1/100th of the serum level although the thyroxine concentration is about 1/25th of serum[13, 0] TBP has also been detected in human and monkey amniotic fluid (Tata unpublished) The detection of thyroxine binding proteins in these extravascular fluids offers no clue to their physiological significance it appears that serum TBP is distributed in extravascular space like other plasma proteins and that extracellular thyroxine is in the main protein bound

(c) Thyroxine binding by Tissue Proteins

As we shall see thyroxine exists in a reversible equilibrium between the extracellular and intracellular binding compartments[534, 751] This entails the presence of thyroxine binding proteins in tissues comparable to TBP in serum Recently the presence of a specific thyroxine binding protein fraction in rat and rabbit skeletal muscle has been demonstrated[15, 3] When skeletal muscle extracts to which were added very minute amounts of radioactive thyroxine were analysed by paper electrophoresis a large part of the radioactivity migrated with a protein fraction which corresponded to the fractions l and m of the globular proteins of Dubuisson and Jacob[406] The electrophoretic mobility of this cellular TBP is very different from that of serum TBP (Fig 6) The binding capacity of cellular TBP is limited compared with that of serum TBP whether it is measured by electrophoretic separation (compare Fig 6 B and C) dialysis or precipitation with trichloroacetic acid The relatively weaker binding has also been observed in the ready displacement of thyroxine from cellular TBP to serum TBP when serum or enriched TBP was added in increasing amounts at a final serum dilution of 1 7 when the serum protein concentration is the same as muscle protein concentration in the cellular TBP extract in the presence of tissue extract almost all the thyroxine was bound to serum TBP It is for this reason that cellular TBP has been more difficult to identify in more vascular tissues such as liver and kidney As with serum TBP the homogeneity of cellular TBP remains unestablished Thyroglobulin itself is able to bind free thyroxine but the bond is weaker

than that of serum TBP[793] Whether this is related to the mechanism of secretion of thyroid hormone from the gland to blood TBP is not known

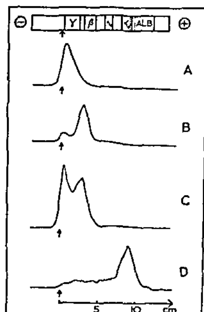


Fig 6

Distribution of radioactivity in electropherograms of ^{131}I labelled L thyroxine added to rat skeletal muscle extract and rat serum The horizontal strip at the top shows the distribution of rat serum α_1 α_2 β and γ globulins and albumin The vertical arrows indicate the points of application of samples A = L thyroxine alone B = muscle extract + L thyroxine ($3\ 5 \times 10^{-8}$ M) C = muscle extract + L thyroxine ($7\ 0 \times 10^{-8}$) D = rat serum + L thyroxine ($6\ 0 \times 10^{-}$ M) (From Tata[1573])

(IV) Physiological Significance of Thyroxine binding

The most obvious and easily acceptable role that can be assigned to protein binding of the circulating thyroid hormones is that it provides a vehicle of transport of the hormones from their site of production to the periphery at the same time it provides a ' buffer to regulate their entry into tissue cells It is not known whether thyroxine and triiodothyronine enter into cells while they are bound to serum TBP[534 751 92] it is much more probable that only the very minute amount of free hormone passes into the cell since it is available to the binding sites on the cell surface Thus the proportionality between the rate of hormonal iodine degradation or utilization and the square of serum PBI derived by Riggs[1305] can be expressed in terms of free thyroxine in the serum in fact the experimental data fit well with this concept Robbins and Rall[13 0] have found a linear relationship when they plotted the rate of organic iodine degradation against unbound serum thyroxine that was calculated

from the values of PBI extrathyroidal degradation rate and BMR provided by Berson and Yalow[166] On this basis one can explain why the BMR (which is proportional to the rate of utilization of thyroid hormones) is increased in hyperthyroidism but not in pregnancy although in both conditions the PBI is elevated In hyperthyroidism the level of TBP remains unchanged from the euthyroid state and hence because of a simple equilibrium phenomenon there is more free hormone available for entry into tissue cells On the other hand in pregnancy the level of free hormone and therefore the rate of transfer into cells is maintained at the pre pregnancy level by an increase in the level of TBP corresponding to the increase in circulating hormone Regarding the cause of this effect of pregnancy it has been suggested[13,0] that the primary effect is a rise in the level of plasma TBP coincident with the rise in other α globulins[998] the increased PBI follows as a compensatory mechanism to maintain the level of free thyroxine so that the normal tissue requirements can be met

Another facet of the physiological significance of circulating TBP is that it offers the most reasonable explanation for the relatively high biological activity of triiodothyronine in most animals This enhanced potency of triiodothyronine corresponds to its more rapid rate of utilization or its biological half life (see Chapter 6) this in its turn is directly related to the greater ease of its entry into the tissues We have already seen (Table I) that triiodothyronine is in fact much more loosely bound to TBP than is thyroxine The argument that a high biological potency may be expected from a thyroxine analogue which is loosely bound to serum TBP and which is therefore more readily available to cellular TBP has received some support Shellabarger[1452] and Newcomer[1151] made the interesting observation that triiodothyronine in the chick had only the same potency as thyroxine whatever the method of assay This prompted Tata and Shellabarger (unpublished) to compare the transport and metabolism of thyroid hormones in birds and mammals They found that the biological half life of thyroxine and triiodothyronine was the same in the chicken in contrast to the much shorter half life for triiodothyronine in mammals Electrophoretic studies of chicken and duck serum showed that there was an absence of any α globulin like TBP corresponding to that in mammalian serum, both thyroxine and triiodothyronine were bound to serum albumin and the binding affinity of albumin for both compounds was the same this again contrasts with binding of the two hormones to mammalian TBP These results support the hypothesis that biological potency of triiodothyronine is dependent among other factors on its availability to tissues as controlled by protein binding in the blood An alternative hypothesis that triiodothyronine is more effective than thyroxine on enzyme systems responsible for the regulation of metabolism has never been proved Why birds should utilize thyroxine as rapidly as triiodothyronine is not

clear, they may have a greater need of thyroid hormones than mammals in order to maintain the higher body temperature and pulse rate per unit body weight (see Chapters 5 and 6) The greater diffusibility of thyroid hormones into tissues from fowl blood is compatible with low PBI values reported in this species[10 0]

There is direct evidence that TBP does in fact influence the rate of entry of thyroxine into tissue cells by a simple binding equilibrium Freinkel *et al*[534] have recently studied the penetration or uptake' of radioactive thyroxine by surviving slices of liver, heart and kidney cortex in the presence of enriched human *TBP* and other serum protein fractions they have shown that the uptake of thyroxine by tissue slices was depressed by the addition of thyroxine binding proteins or serum to the medium this depression was proportional both to the affinity of the added protein for thyroxine and to the concentration of the binding protein (Fig 7) further thyroxine already bound in the tissue could be discharged from it

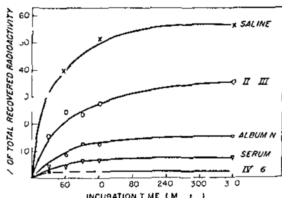


Fig 7

Effect of the addition of whole human serum serum albumin Cohn fractions II-III and IV-VI (thyroxine binding protein) to the saline incubation medium on the uptake of [131]I labelled L thyroxine by calf heart slices The total amount of protein added was the same in all cases (From Freinkel *et al*[534])

by addition of TBP to the incubation medium The reversibility of this phenomenon demonstrates the existence of an equilibrium between cellular and extracellular binding of thyroxine Similar conclusions can be drawn from the work of Beraud *et al*[147] on the inhibition by human serum of the entry of thyroxine into rat kidney slices It is also likely that similar reversible equilibrium between cellular and extracellular binding sites is involved in the uptake of thyroxine and triiodothyronine by erythrocytes and isolated rat diaphragm in the presence of serum[678 332 334 681 333 751] although this has not been unequivocally demonstrated The extraction of a cellular thyroxine binding protein fraction from rat and rabbit skeletal

muscle has made possible the observation of a direct competition between serum TBP and intracellular TBP for the binding of thyroid hormone[1573] (see p. 53). The physiological significance of tissue TBP can only be inferred by analogy with that of serum TBP, and it almost certainly takes part in regulating the entry of the thyroid hormones into cells; it also probably influences the rate of metabolism of these hormones. This last suggestion has been supported by the observation of a progressive inhibition of enzymic deiodination of thyroxine by a muscle preparation to which partially purified muscle TBP was added in increasing amount. The inhibition was proportional to the amount of thyroxine bound to cellular TBP; this means that only "free" thyroxine is susceptible to deiodination.

Myant[1138] has demonstrated that in the intact animal the liver removes plasma thyroxine more easily when it is bound to serum albumin (after saturation of TBP) by "weak" bonds than when it is bound to TBP by "strong" bonds. These studies are of great interest since they show that the conclusions relating to binding equilibria *in vitro* are also valid for the conditions that exist *in vivo*.

(X) METHODS USED IN THE STUDY OF BINDING PROTEINS

The most commonly used method for the separation and identification of thyroxine binding proteins is that of electrophoresis on paper at pH 8.6; at this pH the two major binding proteins in human serum, TBP and albumin, can be separated from each other without difficulty. Both zone and paper electrophoresis have also been carried out at pH 4.5[1318, 534]; this has shown that serum TBP is a more acidic protein than albumin, since TBP exhibits anodic migration while albumin is stationary.

Two methods are in general use for the determination of the binding capacity of a protein for thyroxine or derivatives of thyroxine: (1) increasing amounts of thyroxine (or the derivative) are added to serum containing a trace of ^{131}I labelled thyroxine; after electrophoresis the displacement of radioactivity from the original binding protein can be measured[1318, 1319]; (2) an isolated serum or other protein is added to a sample of serum in which the level of thyroxine (^{131}I and carrier) is so high that the hormone will be distributed between TBP and albumin; a redistribution of thyroxine will then take place depending on the relative affinities for thyroxine of TBP, albumin and the test protein[531]. Robbins and Rall have described difficulties in estimating the saturation of TBP by this method, which Robbins has partially overcome by "reverse flow" electrophoresis.

In human serum the separation of TBP from albumin is relatively simple; this is not the case in rat serum where TBP appears to migrate with albumin[1318]. However, Myant[1138] has shown that these proteins can be separated in rat serum by altering the pH at which electrophoresis is carried out.

One of the major disadvantages of paper electrophoresis in the determination of the binding properties of proteins is that paper itself possesses the property *of binding thyroxine and thus competes with the proteins* under investigation This phenomenon is particularly noticeable when dilute protein solutions such as CSF and amniotic fluids are being tested Another disadvantage of paper electrophoresis is the limited resolution of the proteins this is restricted to the major groups (albumin and the *globulins) hence any binding zone may contain a number of protein* fractions

Recently Rich and Bearn[1 99] have used starch gel electrophoresis[1496] to study thyroxine binding in human serum in contrast to the results obtained with paper electrophoresis they found that about 60% of thyroxine was *bound to the rapidly migrating pre albumin fraction These* experiments have been of a qualitative nature but the method appears to be promising for the purification of TBP, and may have advantages over methods based on fractional precipitation of proteins or electrophoresis on paper or cellulose columns

A new *method for studying the binding of thyroxine by proteins has* recently been developed (Tata unpublished) This method is based on a hitherto undescribed property of thyroxine triiodothyronine and other iodophenols when thyroxine is transferred from an organic solvent to an aqueous medium the dissociation of the phenolic group is accompanied by a *transient apparent deiodination (as seen by loss of radioactive* thyroxine) which can be demonstrated by chromatographic analysis This deiodination is eventually self reversible but the recombination can be accelerated by the addition of certain proteins to the aqueous medium further the presence of these proteins in the aqueous phase *inhibits deiodination of thyroxine when it is transferred from* the organic solvent This inhibition of deiodination is directly proportional to the thyroxine binding affinity of the protein and to its concentration thus graphical analysis of the percentage inhibition of deiodination as a function of protein concentration will give the relative binding affinity and the minimal concentration required for binding This method is much more sensitive than paper electrophoresis and can therefore be used for the estimation of binding power in a dilute protein solution it can more over be used in a physiological pH range thereby avoiding the possibility of artificial binding This method does not however entail the separation of protein fractions and can only be used for the determination of binding affinity of previously purified proteins or for the comparison of binding affinity in different sera or protein solutions

CHAPTER 5

PHYSIOLOGICAL ACTIONS OF THYROID HORMONES

GULL S[853] report in 1874 On a cretinoid state supervening in adult life in women ' is the first description of a condition which resembled cretinism but only came on in adult life Fagge[463] had suggested that endemic goitre might be associated with changes in the thyroid gland but it was not until 1883 that its true association with Gull s disease was really demonstrated In that year Kocher[8 0] of Berne described the changes occurring in patients a few months after removal of their thyroids the symptoms observed loss of energy mental dullness a hoarse voice a thick dry skin swelling of the face and hands and loss of hair were the same as those described by Gull In the same year the brothers Reverdin[1298] also noted these changes in patients who had undergone thyroidectomy These discoveries were followed by investigations into the effects of thyroidectomy in experimental animals (see [691]) which laid the foundation for a more rational approach to the study of the physiological actions of the thyroid gland

In 1895 Magnus Levy[1011] made the classical discovery that the respiratory exchange was depressed in Gull s disease (named myxoedema by Ord[1176] in 1878) and elevated in Graves disease Since then thousands of reports have been published on the functions of the thyroid gland Many of these describe effects obtained with very large doses of thyroid substance or thyroxine It is only recently that workers in this field have recognized what minute amounts of the thyroid hormones are secreted by the gland and will produce responses when administered to humans and experimental animals For this reason emphasis will be laid on work which deals with physiological rather than pharmacological effects

The physiological actions of the thyroid hormones have been chiefly studied from observations on metamorphosis in amphibia growth in mammals effects after removal of the thyroid gland effects on metabolic rate or oxygen consumption in mammals and on the function of other glands or organs Two salient facts which emerge from these studies are (1) the differences of thyroid hormone activity in different species and (2) the dependence of thyroid hormone action on the general status of the animal that is being studied The interrelationship between the thyroid and other endocrine organs in particular the pituitary gland has been discussed already

(I) Calorigenic Action and Thermoregulation

The calorigenic action or effect on cellular oxidation of the thyroid hormones is their most fundamental action in adult mammals and is used as one of the indices of thyroid activity in man The calorigenic action is generally represented in terms of the basal metabolic rate (BMR) this is a measure of heat production in a fasting subject who (ideally) is mentally and physically relaxed Since calorimetry is not usually feasible, oxygen consumption is measured and heat production is then calculated The BMR must be distinguished from total metabolic activity since only the former is influenced directly by the thyroid hormones In clinical practice the BMR of patients is usually expressed as the percentage deviation from the normal range The normal average standard most generally used is that proposed by Aub and Dubois[79] in 1917 Skanse[1477] has criticized this standard and a new lower one has been proposed for Britain[13, 9, 339] a lower standard for Japan has also been published[114, 11, 9]

Early work on the calorigenic action of the thyroid hormone the methods for studying it and the clinical use of BMR determinations have already been reviewed[10, 4, 198, 691, 405, 1404, 140, 1407, 5, 3, 838] and will not be considered here

Pulse rate is sometimes used as an index of metabolic activity but is not generally considered reliable In small laboratory animals the survival time in a closed vessel has also been used for the assay of thyroid preparations and thyroid hormone analogues (see Appendix)

Magnus Levy in his first paper on the effect of thyroid feeding on oxygen consumption in humans[1011] recorded that a basal rate of oxygen consumption of 2 94 cm^3/kg in a subject weighing 77 kg rose to a value of 3 5 cm^3/kg when his weight had fallen to 73 kg after feeding with a thyroid preparation Later Plummer[1235] used thyroxine to stimulate metabolism and estimated that about 1 mg of thyroxine daily was required to maintain the metabolic activity of normal individuals it has since been found[1404, 1405] that Plummer s value is too great and that 500 µg of thyroxine suffices for normal metabolic activity in man

Boothby and his associates[195, 198, 196] first put the relationship between thyroid hormone administration and response on a quantitative basis when they showed that for each milligram of thyroxine injected intravenously into patients with myxoedema approximately 1008 calories were produced the effect was much greater than that obtained with adrenaline which only gave rise to the production of 50 cal/mg injected The strict experimental standards laid down by Boothby have rarely been surpassed and measurement of oxygen consumption has since been established as a sensitive technique for the standardization of substances related to the thyroid hormones in man and other animals

The BMR in hypothyroidism myxoedema and in thyroidectomized animals is lower than that found in normal subjects Conversely in conditions where excessive amounts of thyroid hormone are secreted as in thyrotoxicosis or after administration of thyroid hormones in any form the BMR is raised often to very high levels In this connection the work of Elmer and Scheps[130] and of Salter[1105] should be mentioned this concerns the relationship between blood iodine levels and metabolic activity In myxoedema metabolic activity and blood iodine content are low in hyperthyroidism they are both raised

After the administration to animals of single doses of desiccated thyroid and thyroxine there is an initial period during which no metabolic response is observed (latent period) The BMR then gradually rises to a peak value where it remains for several days and thereafter gradually falls to its initial value Other hormones (insulin adrenaline etc) exert an immediate effect when injected into man and experimental animals The latent period of action of thyroxine has for many years made workers in the thyroid field speculate whether thyroxine was indeed the compound which exerted the gland s effect in the peripheral tissues and a search has been made for derivatives of thyroxine which might produce an immediate response At one time triiodothyronine was considered as a candidate for the title of the peripheral hormone since in man its latent period of action is much shorter than that of thyroxine This is seen in Fig 8 taken from

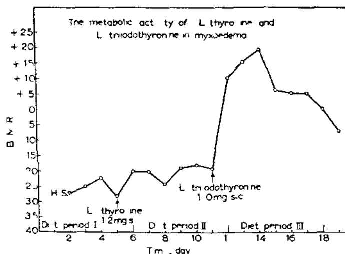


Fig 8

Effect of L thyroxine and L tri odothyronine administered subcutaneously on the basal metabolic rate of the same pat ent with myxoedema (From Asper *et al* [65])

Asper *et al*[65] It will be seen that thyroxine produces a slow response which is maintained for 2-3 weeks A molecularly equivalent dose of triiodothyronine produces its maximum effect in 2 days the effect diminished with equal rapidity and was almost gone 1-2 weeks after the injection It should be emphasized here that the potency of triiodothyronine in eliciting a metabolic response in man is roughly the same as that of thyroxine if considered in terms of the overall response (area under the two curves) obtained The only startling difference is the very rapid onset of action after triiodothyronine injections The difference between the latent periods of action of thyroxine and triiodothyronine has not been observed in thyroidectomized rats[645] The significance of the latent period of action of the thyroid hormones will be discussed later in more detail When assayed by other methods (see Appendix) triiodothyronine has been found to possess about five times the potency of thyroxine[641, 644, 1006, 646, 65, 299, 1053, 72, 1, 75, 951, 363, 1387, 1388, 1456]

The calorigenic action of thyroid hormones observed in the intact animal is also demonstrable in excised tissues obtained from animals with experimentally induced hyperthyroidism Rohrer[1373] first demonstrated an increase of oxygen consumption in liver kidney and muscle isolated from mice which had been treated with desiccated thyroid Conversely tissues from thyroidectomized animals showed a depressed respiration[399, 2, 48, 1055, 1332, 575, 1020, 10, 1, 1010, 1485, 1268, 5, 103, 114, 116] In the earlier work discrepant results were obtained this is probably due to the fact that toxic effects rather than stimulatory ones result from the administration of large doses of thyroid or thyroxine[89, 1658]

Differences in the metabolic responses to thyroid stimulation of these isolated tissues have been observed, depending on the species studied and the thyroid preparation used these differences are particularly marked in tissues such as brain spleen and the gonads[1031, 93, 1385, 1010, 1510, 602, 25, 467, 116, 743, 104] Triiodothyronine has only a higher potency than thyroxine when the oxygen consumption of excised tissues is studied[1735]

In contrast to the response of isolated tissues to thyroid hormones when they are administered to the intact animal is the lack of response of these tissues when the hormones are added to the incubation medium after excision[1386, 1725, 17, 6, 1735, 1, 03, 108, 593, 1761, 1702, 1601] This makes it difficult to explain the thyroid hormone s calorigenic action on the basis of a modification of enzyme systems *in vitro*

Many factors modify the effect of thyroid hormones in the intact animal (see [198]) The state of health of the animal nutritional factors and effect of other endocrine secretions all play a part The mutually potentiating calorigenic actions of thyroxine and adrenaline for example have long been recognized[1024, 1554] the antagonistic actions of thyroid and corticotrophic secretions have also been observed[1091, 385, 24]

Besides the hormonal interactions some endocrine secretions have an independent calorigenic action Evans *et al* [460] have recently studied the calorigenic action of a number of hormones in thyroidectomized and hypophysectomized rats and have confirmed the earlier observations that adrenocorticotrophic hormone has some calorigenic action[73 69 70] Similarly lactogenic and growth hormones have also been reported to have calorigenic activity[461 771 556 1304] However these pituitary trophic factors whether they act directly on peripheral sites or via their target organs only exhibit a small fraction of the calorigenic activity of thyroid hormones Many of the above experiments could be criticized on the grounds that contamination of pituitary trophic hormones with thyrotrophin and completeness of thyroidectomy were not considered

Among the several hundred non endocrine factors affecting basal metabolism that have been described age sex environmental temperature, dose of thyroid preparations and activity of the central nervous system are important From a survey in Britain of the basal energy output of 987 healthy males and 1323 females aged between 3 and 80 years Robertson and Reid[1329] found that the BMR fell sharply with increasing age up to 40 years but after that age little change was observed (see also [79 197]) The high BMR values found in young children reflect a raised activity of the thyroid and are accompanied by increased thyroidal iodine uptake and hormone production[1168 361] Robertson and Reid[1329] have also evaluated the sex difference in BMR at different ages in agreement with earlier work they found that females have a lower oxygen consumption than males Prolonged fluctuations in environmental temperatures are reflected in the basal metabolic activity of small animals but seasonal variations in the human BMR have not been observed by all investigators Gessler found that his own metabolic activity showed seasonal variations but early surveys in American populations demonstrated that the BMR is almost constant throughout the year[142 1814 405 1 53] In Japan however several investigators have noted a significant seasonal fluctuation of BMR in adults[1475 795 1179] Fukuda[519] believes that this fluctuation reflects the low standard of domestic heating in winter

Certain drugs are known to interfere with the calorigenic action of thyroid hormones among these the antithyroxine compound *n* butyl-4 hydroxy 3 5 diiodobenzoate (BHDB) depresses the action of thyroxine on oxygen consumption in small animals[1721 172 112] it has little effect in hyperthyroid humans[5 8] The adrenergic blocking agent Dibenzyline [*N* (2 chloroethyl) *N* (1 methyl 2 phenoxyethyl) benzylamine hydrochloride] has a similar effect to BHDB according to Holtkamp and Heming[754] Schwarz *et al* [1436] however have found that Dibenzyline potentiates the action of thyroxine in rats this resembles the synergism between thyroxine and adrenaline Reserpine also antagonizes the action of

thyroxine and triiodothyronine on oxygen consumption in man and the guinea pig possibly at a peripheral level[893, 358, 359]. Moncke[1093] has in fact treated hyperthyroid patients with reserpine and observed a marked fall in BMR, often to almost normal values. Low oxygen tension has been reported to affect the BMR and Milcu *et al.*[1085] observed a lowering in the BMRs of hyperthyroid patients when they were taken to high altitudes.

Comparative Aspects

Studies on the calorigenic effects of the thyroid hormones have shown that the metabolic rate of any animal is related to its body weight and can be expressed as $M = bW^{\alpha}$ or $\log M = \log b + \alpha \log W$, where M is the metabolic rate per unit time, W is the body weight and α and b are constants. The metabolic rate can also be expressed in terms of the pulse rate which closely corresponds to 0.75 power of body weight[784, 160, 159]. The relationship between body weight, heat production and the rate of utilization of thyroxine is shown in Fig. 9.

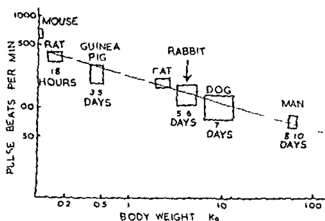


Fig. 9

The relationship between the rate of peripheral utilization of thyroxine and the allometric dependence of pulse rate on body weight in some mammals. In general the pulse rate is proportional to the basal metabolic rate; the allometric relationship is modified from Bertalanffy[159]. The values of 18 hr, 3.5 days, etc., are the average figures for the biological half-lives of thyroxine in the different species derived from literature quoted in the text.

The thyroid hormones do not appear to exert any calorigenic activity in lower vertebrates although thyroxine and triiodothyronine have been identified in some species. Most investigators have failed to observe either stimulation of oxygen consumption by thyroxine or depression of oxygen consumption by antithyroid drugs in frogs and fishes[724, 763, 40, 1378, 457, 715, 1188, 916, 1018], though a few have obtained a positive response to thyroid hormone and antithyroid compounds in fishes[1489, 1487]. Smith and Brown

believe that the failure to obtain responses to thyroid hormones was due to the fact that mammalian thyroid preparations were used, and Matty[148] has found that the parrot fish exhibited greater stimulation by thyroid from the same species than from a commercial thyroid preparation. Fromm and Reineke[45] have thyroidectomized the rainbow trout, which has a diffuse thyroid, with [131]I and have shown that oxygen consumption in this species is unaffected by the thyroid status. A similar conclusion was reached by Matty in his study of the trout [illegible].

E 4. Temperature and Thermoregulation

The physiological regulation of body temperature in homeotherms is the result of a complex interplay between neural and endocrine factors among which the thyroid has an important role. The thyroid hormone acts by stimulating heat production if the environmental temperature is lowered. The function of the thyroid gland in thermoregulation of both homeotherms and hibernating animals has been reviewed from different angles by [illegible] and by [illegible] and Lachance.

Disturbances of the heat-regulating mechanism were considered as one of the most prominent symptoms of myxoedema by the earlier workers, and it has been repeatedly demonstrated that thyroidectomized animals cannot survive in the cold. Detailed studies on the survival time of rats treated with thiouracil have been made and the increased mortality rate in chilled animals has been observed. The increase in metabolic activity has been shown to accompany increased thyroid weight, cellular hypertrophy and raised [131]I uptake by the thyroid together with a raised circulating hormone level. It has also been shown that a raised environmental temperature decreases the body's requirement for thyroid hormone; rats given thyroid powder daily and kept at [illegible]°C survived a shorter time than control animals kept at [illegible]°C or below.

The two- to three-fold rise in the BMR produced by sudden exposure to temperatures near the freezing point is partly due to muscular activity and partly to increased adrenaline secretion. Swanson has postulated that the chief role of thyroxine in acclimation to cold is the potentiation of the calorigenic action of adrenaline rather than a direct action. Eisen and Gross also conclude that the amount of circulating thyroxine is not directly related to the metabolic response to cold. It appears that different mechanisms are involved in the stimulation of heat production depending on the duration of cold exposure of short or long duration. It must be emphasized that the endocrine control of thermoregulation is not the only mechanism involved; although the sympathetic nervous system has no part in controlling the response of the BMR to thyroid hormones, the central nervous system is to some extent

involved It appears therefore that hormonal (chemical) and neural (physical) actions must be considered to act in conjunction with each other

The relationship between thyroid hormone secretion and thermo regulation is of particular interest in the study of hibernating and migrating animals but only scant information is available Work on the hamster garden dormouse marmot and hedgehog shows that in contrast with permanent homeotherms the thyroid exhibits little evidence of hyper activity when exposure to cold occurs during the animal s period of activity in summer[54 1761 837 352] Kayser and Aron[837] have explained this lack of response by suggesting that the seasonal cycle of activity of endocrine glands in hibernating animals cannot be overcome with the stimulus of cold In the garden dormouse (*Eliomys quercinus* L) it has been shown that the thyroid gland continues to function during hibernation but that its activity is accelerated in February and March[898] However, the thyroid itself does not appear to influence the hibernation cycle since Uiberall[1657] has shown that hibernation occurs in thyroidectomized hedgehogs

Little work has been done on thyroid function in migratory birds but Oakeson and Lilley[1163] have found that the thyroid gland of the Gambel s sparrow is most active at the end of the spring and autumn migrations

(II) Growth Maturation and Differentiation

Some workers have interpreted the great variety of physiological actions of the thyroid hormones as a multiplicity of manifestations of a single action on energy metabolism However a growing belief is shared by many that the thyroid hormones have a specific action on growth processes independent of their function as metabolic regulators since substances such as 2 4 dinitrophenol and adrenaline which have calorigenic activity have no effect on growth In warm blooded vertebrates it is not possible to study developmental and metabolic functions independently but it has been shown that in lower vertebrates the thyroid gland has a much greater effect on growth and differentiation than on oxygen consumption In these processes one must constantly be reminded that the thyroid hor mone acts in conjunction with the growth or somatotrophic hormone

(a) *Growth in Higher Vertebrates*

It has been known for nearly 70 years that full body growth to normal adult dimensions is not attained in athyreotic animals or in animals which are thyroidectomized during the early period of development The most striking example of this is the dwarfism of the congenital hypothyroid child or cretin Early trials of replacement therapy in cretins showed that growth can be restored to some extent The amount of thyroid hormone administered has also been found to be of paramount importance and excessive doses will inhibit growth as they inhibit metabolism[1683] Cameron and Carmichael[252 253] administered large doses of thyroxine to rats and

found that growth as a whole was retarded because of the stimulation of catabolic processes on the other hand differentiation was stimulated and resulted in hypertrophy of several organs notably the heart liver kidneys and adrenal glands Cameron and Carmichael[254] used the decrease in growth rate of rats as a method of assay of thyroid hormone this method of bioassay is however of limited value since the relationship between diminished growth rate and dose of hormone is only very roughly quantitative[855] Certain vitamins antibiotics lipids and liver extracts have been shown to protect animals against weight loss and death resulting from extreme hyperthyroidism[1431 1555 43 1246 1069 413 415 416] Stevens and Henderson[1512] have recently described a method for the assay of the factor in liver based on its protective action against weight loss in hyperthyroid rats

Stimulation of growth by small doses of thyroxine in thyroidectomized rats has been demonstrated by Rowlands[1394] and by Simpson *et al*[1470] the growth promoting action of triiodothyronine has also been demon strated[644] as has its growth inhibiting activity when given in too high a dose (Fig 10) This extreme sensitivity in the response of body weight

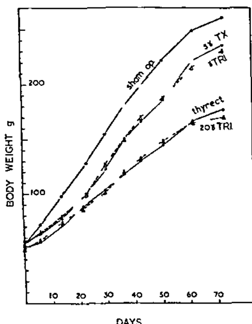


Fig 10

Effect of thyroid hormones on total body growth in young thyroide tomized rats TX = thyroxine TRI = tri odothyroxine thyrect = thyroidectomized controls sham op = sham-operated control One microgram triiodothyronine/day and 5 µg thyroxine/day stimulate the reduced growth rate in thyroidectomized animals while 20 µg triiodothyronine/day reversed this effect (From Gross and Pitt Ri ers[4 4])

to overdosage with thyroid hormone probably explains the failure of certain workers to observe its growth promoting action in certain instances [871, 1647, 1206]

It has now been established beyond doubt that the thyroid hormone is essential for growth during early life in man monkeys ruminants rodents and birds[673, 1, 88, 1196, 798, 1632, 1565, 488, 489, 1716, 681, 1, 17, 1474] Further differences in growth rates of different strains of lambs and chicks have been correlated with thyroid secretion rates the faster growing strains have an inherently higher thyroxine secretion rate than the slower growing strains[1474, 681]

Besides total body weight the growth of certain tissues is also markedly retarded in hypothyroidism Alterations in skin and hair growth are evident in myxoedema and have been invoked in the diagnosis of hypothyroidism[1061] In young sheep a poor condition of the fleece has been found to accompany stunted growth after thyroidectomy [17, 14, 3, 1031] Thyroidectomy has recently been shown to prevent maturation of secondary wool follicles but the condition can be corrected by the administration of thyroxine[479] In chickens thyroidectomy results in retardation of feather development while feeding small amounts of thyroid substances or iodinated proteins stimulates feather growth in growing birds[178, 1196, 798, 1647] Large doses of thyroid hormone administered to mature birds cause abrupt moulting of old feathers or barbulation and depigmentation of new feathers which appear[1765, 1767, 1033, 78, 6, 5] Thyroxine and related compounds are capable of reversing the effects of thyroid deficiency on fowl plumage[1187, 435, 39]

The mechanism of growth stimulation remains unknown whether the action is secondary to a stimulation by growth hormone or is a result of varied but direct actions has yet to be determined The fact that thyroxine has a mitogenic action on rat liver and epithelium of the crypts of Lieberkuhn in the duodenum is of interest[1729, 9, 1] However the finding of Leblond and Carriere[9, 1] that thyroxine increases mitotic rate in thyroidectomized rats only in the presence of the pituitary or growth hormone suggests a secondary (but vital) role for the thyroid hormone in the process of cell division and differentiation and indicates that during development the combined effects of pituitary growth hormone and the thyroid hormone are essential

(b) *Effect on Bone and Tooth Formation*

The thyroid hormone has an important role in osseous development in cretinism or juvenile myxoedema there is marked retardation of ossification and the body retains infantile characteristics of bone structure not encountered to the same degree in other types of dwarfism with retarded

bone age Thyroid treatment causes rapid acceleration of bone development The maturing skeleton in childhood is more sensitive than are other systems to thyroid hormone deficiency Zondek and his colleagues have recently described a case of thyrogenic infantilism in which the patient shows a marked retardation of bone age as also of somatic and sexual development[1772, 1773] These patients show no other clinical signs of hypothyroidism or myxoedema and respond dramatically to thyroid therapy In thyroid deficiency in children the upper and lower skeletal segments remain infantile in relation to the patient s age and there is marked retardation of the ossification of the epiphysis and appearance of bone nuclei together with a lack of maturation of the naso orbital configuration [1716, 1717] see also leading article *Brit med J* 1958 p 567 Wilkins[1715] has stated that the effect of thyroid hormone on cartilaginous ossification is specific in thyroid deficiency this leads to deposition in islets (see Fig 11) Coryn[310] concluded that the action of thyroid hormone

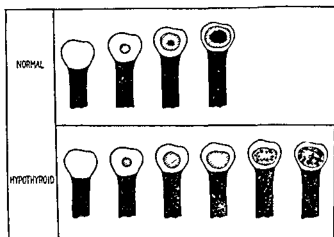


Fig 11

Schematic representation of epiphyseal development in the bone of normal and hypothyroid children The shaded areas represent the change which occurs in the epiphyseal cartilage cells preparatory to calcification The black areas represent calcification and bone formation (From Wilkins[1716])

on the skeleton is complex and influences all phases of endochondrial osteogenesis The correction of retarded bone formation with desiccated thyroid[179] and with triiodothyronine[943] has been demonstrated

In hyperthyroidism radiographic evidence has shown that osteoporosis is common and metabolic studies have related hyperthyroidism to an increased urinary and faecal excretion of calcium and phosphorus[888, 153, 1238, 77, 775, 7, 76, 6] The relatively normal values for serum calcium

phosphorus and alkaline phosphatase found in hyperthyroidism suggest that bone demineralization can be attributed to osteoporosis in these patients Experiments performed with radioactive calcium (^{45}Ca) have shown that in hyperthyroidism bone formation and destruction were proceeding at increased rates[877]

The action of thyroid hormone on bone maturation in other animal species has also been studied In 1894 Hofmeister observed a retarded bone growth and a swelling vacuolization of the cartilage in thyroidectomized rabbits which he described as 'chondrodystrophia thyropriva Later detailed studies on skulls and skeletons of thyroidectomized rabbits and sheep indicated that anomalies in skull development could be partly explained by the relatively slower growth of skull and jaws compared with that of the teeth the locus of damage to both growth and maturation patterns of skeleton is in the diaphysio epiphyseal plane without any modification of bone texture and weight or prolongation of the growth period to compensate for diminished velocity[1623] Similar effects have been described in rats in one instance the bone of a 72 day old thyroidectomized rat had the same appearance as that of a normal animal of 15–20 days[766 138 1161 137 1653 1654 1114] In hypophysectomized rats a virtually normal picture of bone growth is restored by growth hormone administration except that no closure of the epiphysis occurs but administration of thyroxine leads to closure of the epiphysis[64] Becks and his colleagues[137] conclude that in bone, growth hormone stimulates growth only while the thyroid gland controls both growth and differentiation The balance of *control between growth and thyroid hormones and the effect of various* hormonal and dietary influences on total body growth and on the growth of bone and teeth have been recently described and reviewed in further detail[1 18 797 134 989] The extreme sensitivity and dependence of bone growth on thyroid hormones has been brought out by the work of Walker[1690], he has shown that the measurement of skeletal age compared with the chronological age of immature rats gives an accurate indication of the *skeletogenic potency of any substance (Fig 12) By this method triiodo* thyronine was found to be nearly twice as potent as thyroxine but triiodo thyroacetic acid which is metabolically less active than thyroxine had a higher potency in the maturation test

From the work of Fell and Mellanby[472 4 3] it appears that the thyroid hormones may possibly have a direct action on bone growth Cultivation of chick embryonic long bone rudiments in the presence of thyroxine and triiodothyronine *led to two types of effects (1) a stimulatory effect on the* maturation of cartilage more pronounced in rudiments explanted at the blastematous stage (2) a toxic effect causing retardation of growth and cellular degeneration in the older rudiments and during later stages of cultivation The histological effects of triiodothyronine were qualitatively

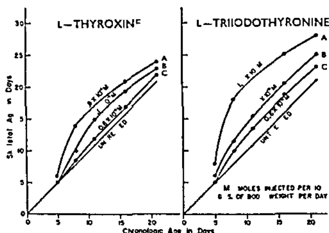


Fig 12

Quantitative relationship between the dose of thyroxine and triiodothyronine and enhancement of skeletal maturation in new born rats A = 1 8 × 10^{-9}M B = 1 1 × 10^{-9}M C = 0 6 × 10^{-9}M (From Walker[14])

similar to those of thyroxine but the inhibitory effect of the former compound was about four times that of thyroxine The effects of thyroid hormones on embryonic skeletal rudiments are to some extent comparable with the effects of hyperthyroidism on the post embryonic skeleton

The effect of thyroid hormone on teeth is comparable to that on bone growth and in cretins tooth development is retarded The thyroid hormone also controls the rate of eruption of teeth this has been particularly well studied in the rat Karnofsky and Cronkite[834] demonstrated that thyroxine markedly accelerates the eruption of teeth in new born rats the time of eruption being dependent on the dose of thyroxine on the other hand thyroidectomy or thiouracil treatment decreases the eruption rate of rat incisors and retards dentine and root development[1771 734 133 559] Garren and Greep[559] Shellabarger[1454] and Walker[1690] have shown that the incisor eruption rate in rats could be successfully used for the assay of thyroxine and similar compounds The activity of the thyroid gland also appears to be directly related to the incidence of dental caries in rats administration of thyroid hormone counteracts the increased incidence of caries produced by radiothyroidectomy[171 1125]

(c) *Effect on Growth and Differentiation in Lower Vertebrates Metamorphosis*

By the end of the nineteenth century much information was available concerning the sequence of events and the changes occurring in tissue structure during metamorphosis in amphibia There was however no clue as to the agent which controls these changes until Gudernatsch[650] made the important discovery that feeding of horse thyroid gland to

anuran frog tadpoles (*Rana temporaria R esculenta Bufo vulgaris*) and urodele larvae (*Triton alpestris*) led to a suppression of further growth and produced an extremely precocious metamorphosis Evidence in support of the hypothesis that the transformation from the larval to the adult stage is effected by the animal s own thyroid was provided by Allen[30] and Hoskins and Hoskins[765] who showed that thyroidectomized larvae failed to metamorphose although they continued to grow Thyroidectomized tadpoles or larvae may grow to a gigantic size these giant tadpoles undergo metamorphosis if fed with thyroid substances[31 1454] Since that time the role of the thyroid gland in amphibian metamorphosis has been more extensively investigated than any other aspect of thyroid function in cold blooded vertebrates and excellent reviews have been written on work done during the last forty years[551 1 96 652 33 34 205 488 995 458]

Later attempts were made to establish a relationship between the activity of the thyroid gland and metamorphic changes In 1919 Allen[32] reported a gradual increase in thyroid size during the early stages of metamorphosis in toad larvae followed by a diminution in size when metamorphosis was at its height Work in several species involving cytological studies of cell height colloid content epithelial mass and intracellular vacuoles has shown that the thyroidal activity increases greatly and reaches a maximum near the time when the tail is undergoing resorption after this there is a decrease in the height of the epithelium and intracellular vacuoles[1049 1481 454 455 111 345 313 1413 900] There have been some discrepancies in the findings of different workers owing to the wide variety of species investigated Sklower[1481] and D Angelo and Charipper[345] observed that the thyroid follicles collapse at the climax of metamorphosis while Etkin[455] found no distinct stage of follicular evacuation Gorbman and Evans[598] have correlated histological differentiation with the beginning of thyroid function in ^{131}I studies in *Hylla regila* but Money *et al*[1096] were unable to detect any difference in ^{131}I turnover during metamorphosis in *Rana pipiens* However Saxen *et al*[1413] studying *Xenopus laevis* have shown unequivocally that both thyroidal and extrathyroidal iodine metabolism correspond with histological changes These authors have used six different parameters for the quantitative measurement of thyroid function and its stimulation by the anterior pituitary gland the results are summarized in Fig 13

Although it is likely that the decrease in thyroidal ^{131}I shown in Fig 13 represents an increased rate of release of thyroid hormones and that the lowering of body $PB^{131}I$ is related to increased thyroid hormone utilization the hormones themselves have only been identified during metamorphosis very recently Shellabarger and Brown (unpublished) have shown that thyroxine and triiodothyronine are present in the thyroid glands of tadpoles during different stages of metamorphosis

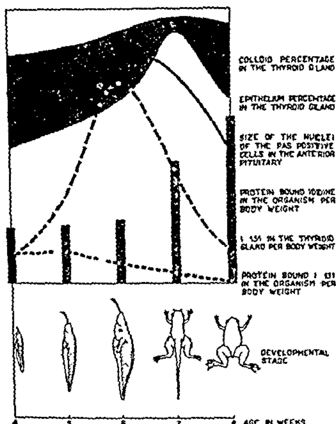


Fig 13

Schematic representation of morphological and functional changes occurring in the pituitary and thyroid gland during the development and metamorphosis of *Xenopus laevis* tadpoles (From Saxén *et al* [1418])

In contrast to the amount of information available on the physiological function of the thyroid hormones in amphibian metamorphosis relatively little is known about their effects on growth and differentiation in other classes of lower vertebrates such as tunicates acraniates and fishes or invertebrates Contradictory findings have been reported but it appears that the effects of the thyroid on growth and differentiation is less spectacular than in amphibians (see [488] [895] for reviews)

None of the theories put forward to explain the mechanism of action of thyroid hormones on metamorphosis has gained wide acceptance The suggestion has been made that the thyroid controls metamorphosis by its influence on metabolism However some investigators failed to observe any increase in metabolic rate during metamorphosis nor could they accelerate metamorphosis by certain agents that increase the metabolic

rate For these reasons this theory is unconvincing Many investigators who did observe an increase in metabolic rates of tadpoles during metamorphosis have expressed the belief that such a rise in metabolism is an accompaniment rather than a cause of metamorphosis as first suggested by Huxley[783] Among other early theories are those of Champy [70] and Aleschin[28] Champy believed that the thyroid hormone causes an increase in mitotic activity in those organs which are characteristic of terrestrial life i e lungs limbs and intestine while it has regressive effects on characteristics of aquatic existence, such as the tail gills and the horny beak Aleschin considers that impregnation of larval tissues with thyroid hormone leads to autolysis especially in the gills and intestine, autolysis would lead to a general acidosis and this would promote further autolysis this would occur even when the thyroid is in a resting state towards the end of metamorphosis The principal objection to this theory is that it only accounts for the disappearance of certain tissues but provides no explanation for the appearance of new ones (limb buds) Lynn and Wachowski[995] conclude that the characteristic responses of different tissues to the hormone result from inherent properties of the tissues themselves This leads us back to the basic problems in embryology of differentiation and determination and we conclude that the diverse changes which occur during metamorphosis are predetermined and are only triggered off by the thyroid hormone

(III) Effect on Water and Electrolyte Metabolism

One of the earlier noted effects of thyroid hormone administration was a marked diuretic action In myxoedema a considerable amount of salt and water was found to have accumulated in the organism and replacement therapy with thyroid caused the loss of extra fluid together with the dissolved electrolytes[1131, 477, 937] It was also shown that the loss of water influenced the volume and properties of the body fluids[3, 6, 1609] The diuretic activity of thyroxine was even observed in diabetes insipidus[560] On general grounds one might expect that hyperthyroidism would be accompanied by dehydration and myxoedema by increased water content however the reverse effect of a considerable concentration of blood in myxoedematous patients was noted by Thompson[1609] further experimental hypothyroidism led to dehydration of blood, skeletal muscle and brain[676] Administration of thyroid extract or thyroxine effected a transfer of water from the tissues to the blood thus increasing the total blood volume by 25–30%[1609, 648] It therefore appears that the diuresis of hyperthyroidism is secondary to dilution of the blood

The *studies* of Byrom[250] on the metabolic balance of electrolytes and proteins helped to clarify the function of the thyroid hormone in water metabolism Byrom found that administration of thyroxine to normal or

myxoedematous humans was followed by losses of sodium and potassium ions during the diuresis which followed There was however a difference in the effect in normal and hypothyroid patients, in the latter the loss of sodium was much greater than the loss of potassium whereas in normal individuals a higher net loss of potassium resulted This indicated that *the fluid accumulated by myxoedematous patients was largely extracellular* but that in normal subjects, thyroxine caused a depletion of intracellular water and salts Boekelman[191] believes that the thyroid gland has an important function in the regulation of body potassium levels The phenomena noted by Byrom occurred only as a result of the large doses of thyroxine administered Boothby and his colleagues failed to observe any consistent effect of small doses of thyroxine either on sodium or on potassium excretion[199] More recently careful metabolic balance studies in humans on the effects of small doses of thyroxine and triiodothyronine also failed to demonstrate a consistent effect on overall balance of sodium and potassium in spite of a very profound diuresis[1275, 65] Rawson *et al*[1275] also observed that an increase in the level of sodium and chloride in the serum accompanied the diuresis this suggested that enough water was lost to cause a relative concentration of extracellular fluid This work failed to demonstrate any effect of thyroid hormones on chloride excretion although earlier studies described changes in chloride excretion accompanying changes in thyroid status[348, 1257] Koivusalo and Pekkarinen[87] showed that the chloride concentration in the sweat decreased with increasing hyperthyroidism

Thyroid hormones also affect the metabolism of calcium and phosphorus but how this is brought about is not clear Many factors control the metabolism of these two elements i e parathyroid hormone calcium intake renal function and vitamin D Reports have appeared describing negative calcium and phosphorus balances in hyperthyroidism in man or during thyroid administration in *man and lower animals*[1191, 77, 40, 13, 6, 1327, 13, 8, 982, 614, 1275, 65] If sufficient calcium is included in the diet a positive balance can be established even though massive doses of thyroid hormone are administered moreover the negative calcium balance occurring in thyrotoxicosis can be abolished by calcium feeding However both Rawson[12 5] *and Asper*[65] *and their colleagues noted little change in calcium* excretion or in serum calcium levels after administration of small doses of thyroxine or triiodothyronine to myxoedematous patients although marked phosphorus excretion was provoked The dose of thyroid hormone administered and the thyroid status of the subject are factors which will affect calcium *retention or excretion in hypothyroid children or cretins* small doses of thyroxine cause a retention of calcium while larger doses produce the opposite effect[1032, 815] The action of thyroid hormone on calcium metabolism differs profoundly from that of parathyroid hormone

with the latter both the serum calcium level and urinary calcium excretion are greatly elevated at the expense of bone calcium When Rawson *et al*[12 5] calculated the phosphorus loss on the basis of nitrogen or calcium loss they found that more phosphorus was lost than would be expected from the ratio of phosphorus to nitrogen in normal skeletal muscle or calcium to phosphorus in bone A similar effect was produced with triiodothyronine and the acetic acid analogues of thyroxine and triiodothyronine[1 61] There was no change in the phosphorus level in serum and the source of this excess phosphorus is as yet undetermined The findings of Greenberg *et al*[615] Lamberg and Ostling[899] and Bastenie and Ermans[129] indicate that administration of thyroid hormones causes a more rapid turnover of phosphorus and an increase in the rate of transfer across cell membranes

Among the ions studied magnesium has attracted some attention Dine and Lavietes[378] proposed that changes in thyroid activity could induce changes in the ionization of magnesium without affecting its total blood concentration in myxoedema all the magnesium was believed to be in an ionized or ultrafiltrable state while a large part of it was bound in normal subjects This hypothesis has not been confirmed[307 311] Administration of large doses of thyroxine has been shown to cause an increased requirement of magnesium[1686] these authors attribute this to an inhibition of the action of magnesium ions in oxidative phosphorylation caused by the chelating action of thyroxine However the large doses of thyroxine and magnesium administered in these experiments preclude their having much physiological significance

(IV) Effect on Nitrogen and Lipid Metabolism

The profound effects of thyroid hormones on such fundamental processes as growth and maturation of tissues and on the release of energy from foodstuffs are bound to manifest themselves in the metabolic equilibrium of almost all cellular constituents The changes in metabolism of nitrogen fat and carbohydrates provoked by thyroid hormones are summarized below and should be considered in the light of these fundamental actions rather than as isolated effects

(a) *Nitrogen Metabolism*

Hadden[654] noticed that in myxoedema the excretion of urea was diminished below its normal level the converse effect appeared in early reports on replacement therapy in hypothyroid subjects[1177 1071] Muller[1128] recognized the increased nitrogen excretion in Graves disease the effect of thyroid hormone on increased protein catabolism was also demonstrated by Magnus Levy[1011] who found that the increase in respiratory exchange in patients with Gull s disease treated with thyroid preparations was

accompanied by an increased urinary nitrogen excretion When creatine and creatinine excretion were studied it was shown that their metabolism was disturbed both in hypothyroidism and thyrotoxicosis[14, 0, 517, 1418] This discovery has been followed by many reports associating thyroid function with nitrogen metabolism as reflected in changes in creatine and creatinine excretion[164, 829, 799, 718, 1190, 851, 1461, 1248, 1611, 90, 91, 1613, 1718, 1402, 1126, 1247, 740, 887] In general secretion or administration of excessive amounts of thyroid hormones leads to creatinuria and an increased serum creatine level concomitantly there is a decreased concentration of creatinine in both urine and blood In hypothyroid children creatinuria which is a normal process in childhood is absent but creatine excretion appears rapidly during thyroid medication In adult subjects with hypothyroidism the deviation from normal creatine and creatinine metabolism is less marked It should not be forgotten however that other pathological conditions such as diabetes liver diseases and muscular dysfunction also affect creatine metabolism and the retention of plasma creatinine in renal failure is well known

The creatinuria observed in thyrotoxicosis is related to thyrotoxic myopathy this was foreshadowed by Askanazy[6] who observed that degenerative changes in skeletal muscle in patients with Graves disease resembled those in muscular dystrophy Dubois[405] explained this metabolic defect of hyperthyroidism by postulating that in subjects with increased oxygen consumption a given amount of muscular work required twice the caloric expenditure required by the normal subject It has been shown that a positive nitrogen balance in hyperthyroidism can be restored if the caloric intake exceeds the basal energy requirement[405, 1407] This effect of thyroid hormone in provoking a muscular wastage and inefficient utilization of protein has been the object of many recent studies in cases of thyrotoxic myopathy[1612, 1769, 1253, 1087, 11, 717, 1681] Vestermark[1681] has emphasized that the difference between creatine metabolism in patients with thyrotoxicosis not accompanied by muscular dystrophy and in thyrotoxic subjects with myopathy is related to the degree of hyperthyroidism In experimental animals the relationship of the thyroid to creatine metabolism is similar to that found in man The creatine and phosphocreatine content of the muscles of the rabbit and rat are increased after thyroidectomy and decreased after the administration of thyroid substance[1693, 1891, 6, 187] Wilkins and Fleischmann[1718] found that administration of thyroxine and thyrotrophic hormone to intact animals caused a loss of stored creatine but was not accompanied by any increase in the rate of creatine synthesis they concluded that in hyperthyroidism there is defective transformation of creatine to creatinine and conversely that increased creatine creatinine conversion takes place in hypothyroidism

Thyroid hormone produces a response in metabolism which is

proportional to the dose administered, this led Peters *et al*[1 14] to adopt the creatine tolerance test[1613] for clinical purposes More recently, Kuhlback[887] has found that the plasma creatine creatinine ratio is a useful diagnostic aid as an index of peripheral action of thyroid hormones in thyrotoxic patients and is superior to BMR measurement in these patients

Little is known about the mechanism of the defect in creatine metabolism in thyrotoxicosis or experimental hyperthyroidism Shorr *et al*[146] suggested one year before Lohmann[986] discovered creatinephosphokinase that the thyroid hormone interferes with the enzyme system cataly ing the phosphocreatine ⇌ creatine reaction Later Askonas[63] found that thyroxine had a strong inhibitory action *in vitro* on purified creatinephosphokinase and suggested that this might represent the mechanism of thyroid hormone action on nitrogen metabolism An alternative mechanism might be put forward involving a modification of tissue permeability to creatine In metabolic studies with labelled creatine in cases of progressive muscular dystrophy Roche *et al*[13 0] and Benedict *et al*[144] have shown that creatine, once it is synthesized cannot enter muscle cells but is instead excreted in the urine In this disease there is a defect in creatine metabolism which resembles that in thyrotoxicosis Recently Kuhlback and Wahlberg (see [887]) have observed that creatine is not absorbed and metabolized by muscle in the normal way in thyrotoxic patients the greater part of a dose of labelled creatine was excreted within 24 hr this may explain the diminished creatine tolerance in thyrotoxicosis

The catabolic action of thyroid hormones in protein metabolism is indisputable these hormones also possess anabolic activity when they are administered to hypothyroid subjects these anabolic effects were found to be particularly striking when hypothyroidism was corrected by substitution therapy in children[807 81] The apparent paradoxical action of thyroid hormones as protein catabolizers in hyperthyroid adults and as protein anabolizers in the growing animal may be due to the synergistic action of thyroid hormones and other hormones such as growth hormone or the androgens Whether the thyroid hormones provoke anabolism or catabolism may also depend to a large extent on the dose administered and on the metabolic state of the animal In thyrotoxic adults the negative nitrogen balance is converted by administration of testosterone to a positive balance without reduction of the high basal metabolic rate[855] The dependence of nitrogen balance on dose of thyroid hormone has been demonstrated in a study of the rate of protein synthesis from [^{15}N]glycine in patients with primary myxoedema and normal volunteers before and during administration of triiodothyronine[335] It was shown that in hypothyroid patients triiodothyronine restored the low rate of protein synthesis to a normal level whereas in healthy volunteers it depressed the protein synthesis rate Crispell and his colleagues[336] have also shown that a large

dose (100 μg) of triiodothyronine daily produces negative nitrogen balance in myxoedema

In myxoedema the excess of protein in the extracellular fluid has been termed deposit protein' Increased nitrogen excretion caused by thyroid hormone administration to myxoedematous patients has been mainly attributed to the catabolism of this deposit protein [199 1407], which appears to be in the globulin fraction[376 1684] However there is considerable disagreement concerning the level and distribution of plasma proteins in experimental hypothyroidism For instance it has been observed that thyroidectomy causes an increase in total plasma proteins of rat blood particularly in the α globulin fraction[963 918 231] the opposite effect has been reported for total plasma protein[7] Results which were difficult to interpret were also obtained by Nihei[1158] Agnoletto and Agnoletto[14] and Cama *et al* [251] in studies on the level and distribution of plasma proteins in hyperthyroid rats and rabbits In humans findings are more consistent in myxoedema the ratio of extravascular to intravascular distribution of ^{131}I labelled albumin is increased from the normal ratio of 60 40 to 73 23[180] thyroid medication causes a decrease in extravascular albumin [1435 962 139] Rothschild *et al* [1392] have made the further interesting observation that thyroid hormone enhances albumin production in the liver to a level which nearly compensates for the increased albumin utilization as a result no appreciable negative albumin balance takes place

(*b*) *Lipid Metabolism*

As is well known the concentration of cholesterol in the serum of man the dog rat rabbit and fowl is elevated in hypothyroidism and after thyroidectomy in hyperthyroidism and after thyroid administration the blood cholesterol level is however only slightly below the normal value [441 208 778 1425 580 490 1608 1 10 500 682 1083 1213 375] Among the lipids cholesterol shows the greatest regularity in response to the circulating level of thyroid hormone but other lipid components are affected similarly Thus the levels of neutral fat phospholipid β lipoproteins some fatty acids total free tocopherols and the electrophoretically immobile chylomicron fractions have all been shown to vary inversely with thyroid activity or circulating hormone level[208 580 1210 500 1169 610 1241 499 1546]

Peters and Man[1 10] however have been unable to confirm the report that neutral fat decreases more than does cholesterol in hyperthyroidism[208] and find no correlation between the response to therapy and blood neutral fat either in myxoedema or thyrotoxicosis The variation in serum cholesterol levels in any normal population is large and is accompanied by a corresponding fluctuation in the levels of other lipid fractions The distribution of lipid components in uncomplicated hypothyroidism is normal[1210 1212 1 13] but is disturbed in other types of hyperlipaemia

especially in those caused by liver diseases, fractionation of serum lipids would therefore provide more information in the diagnosis of hypothyroidism than measurement of total cholesterol alone.

The level of fat in the diet plays a major role in the development of hypercholesterolaemia in certain species. Malnutrition or starvation causes serum lipids to fall[439, 853] and it has been shown that partial starvation in rats prevents the hypercholesterolaemia of hypothyroidism[138]. In both the rat and rabbit high cholesterol and high fat diets provoke hyperlipaemia[1651, 616] and it has been shown that in rats a diet rich in fat or large doses of cholesterol will protect animals against the lethal effects of large doses of thyroxine[448, 816]. However in man and dog it is difficult to alter the blood concentrations of lipid or the normal interrelationships of lipid components by dietary variations though the fat stores in the body may be affected[1652, 1535, 1534, 853].

Since thyroid hormones cause a greater expenditure of energy, the loss of fat stimulated by thyroid hormone administration may to some extent be the result of a non specific wasting effect. However Abelin and Kursteiner[4] obtained evidence that thyroid secretion has a direct action on fat metabolism: they showed that a large proportion of the body fat of rats disappeared within 16 hr of administration of large doses of thyroid; this was before any general metabolic effect of the hormone was detectable. Moreover the stimulation of BMR by dinitrophenol without a concomitant decrease in serum cholesterol[34] provides further evidence in favour of a direct action of thyroid hormones on fat metabolism.

The mechanism of this action has been partially elucidated in recent years: it appears that the thyroid hormones influence lipid synthesis and degradation rather than absorption and digestion of fats. In rats thyroid status influences neither intestinal absorption of cholesterol[1381] nor the rate of passive diffusion of cholesterol out of the plasma[543]. However Chanda and his colleagues have shown that thyroid substance enhances both the rate of absorption and the digestion of carotene in cows and goats; thiouracil administration retarded both processes[71, 27].

The effects of thyroid hormones on fat metabolism in the liver have received much attention. Thyroid deficiency produced by thyroidectomy or thiouracil administration results in a marked increase in cholesterol content of the liver but in only a slight increase in total fat, while hyperthyroidism diminishes the cholesterol content[68, 1460]. Fatty livers in rats appear to protect these animals against hyperthyroidism[683]. Further Scaife and Migicovsky[1414] have shown that liver homogenates from thyrotoxic rats exhibit a marked reduction in their ability to synthesize cholesterol. However later work on the incorporation of deuterium and tritium into cholesterol showed an increase in total body cholesterol synthesis in hyperthyroidism[1044, 138]. This discrepancy is probably due to the fact

that in experiments *in vitro* factors such as biliary and faecal excretion are not involved. Reed *et al*[1279] found that high doses of thyroxine caused a marked reduction in the total fat laid down without altering the distribution of fat depots but later work by Mackay and Sherrill[999] has shown that the total fat content of adult rats fed on a diet rich in fat was actually decreased after thyroidectomy. Tuerkischer and Wertheimer[1616] studied the deposition of glycogen in adipose tissue and concluded that thyroxine accelerated the conversion of carbohydrates to fat. Friedman *et al*[542] have shown that in the rat hyperthyroidism raises the sterol secretion rate and the biliary secretion rate of cholesterol above the values observed in normal animals; in animals made hypothyroid by thiouracil these secretion rates were reduced. Hyperthyroid rats were also able to eliminate administered hypercholesterolaemic serum faster than normal rats; the elimination was slowest in hypothyroid animals. Ligation of the bile ducts caused a marked rise in serum cholesterol of the hyperthyroid rats but only a slight rise in thiouracil treated animals. The excretion of bile acids is also influenced by the thyroid gland. Both cholic acid and chenodeoxycholic acid are secreted at a slower rate in hypothyroid rats[1407, 442, 1697]; in hyperthyroidism the excretion of bile acids is not greatly affected but their composition is changed, resulting in a decrease of taurocholic acid and a rise of taurochenodeoxycholic acids[442]. The thyroid hormone appears to have similar effects on the metabolism of phospholipids; their synthesis in the liver is increased in rats treated with thyroxine and decreased in thiouracil treated animals, although the actual amount of phospholipid phosphorus is greater in livers of hypothyroid animals[495]. We may therefore conclude that the thyroid hormone accelerates the rate of synthesis of cholesterol and the phospholipids as well as their rate of breakdown and excretion. The consistent rise in serum lipids observed when thyroid function is depressed probably results from a greater retardation of lipid degradation than of lipid synthesis.

(V) Effect on the Central Nervous System

The role of the thyroid gland in the development and function of the central nervous system has been particularly emphasized in the literature[691, 1, 14, 1717, 141, 1064, 1056]. Probably no tissue suffers more severely than the brain from a lack of thyroid hormone during foetal development and in early life. The changes that occur in the central nervous system as a result of thyroid hormone deficiency vary according to the time of onset of the disease. In the cretin the retarded mentality results in loss of intellect, whereas in adult myxoedema the symptoms are less severe and appear as reduced mental alertness rather than absolute mental incapacity. Furthermore, cerebral function can usually be restored by replacement therapy if thyroid deficiency appears in adult life but is not restored by

such treatment in congenital hypothyroidism As will be seen later, the thyroid hormone possesses the specific property of controlling maturation of the cerebral cortex during a critical phase of development

Changes in cerebral maturation have not been extensively studied in cretins but considerable information is available on experimental cretinism in laboratory animals Marine and Lenhart[1028] produced symptoms resembling cretinism in puppies born of thyroidectomized bitches maintained on an iodine deficient diet Hammett[674, 675] showed that thyroid deficiency in young rats resulted in brain tissue which had a lower water content than normal brain More recently cretinism in young animals has been induced by treatment of the mother with thiouracil or by surgical or radiation thyroidectomy of the new born The brains of these animals show morphological changes such as delayed appearance and diminution of myelination in fibre tracts and a retardation of general maturation of nerve cells[1] a decrease in size of cortical neurones[419] a decreased rate of brain growth and a reduction of the number of axons in the cerebral cortex[417] Changes in the cerebral cortex of rats thyroidectomized at birth could be reversed if thyroid therapy was started by the twenty fourth day after birth[758] The effect of thyroid hormone deficiency on brain development is the same as that produced by inanition[417, 58] It is concluded that in the absence of thyroid hormone, the neurones are unable to absorb the nutrients transported in the blood stream which are essential for protein synthesis this would provide an explanation for the irreversibly retarded mental development in cretins

The effects of thyroid therapy on the structure and organization of the developing cerebral cortex in hypothyroidism have recently been studied at the enzyme level Hamburg and Flexner[667] analysed the succinic dehydrogenase cytochrome oxidase and aldolase content of the frontal cerebral cortex of young rats and found that the activity of these enzymes begins to increase rapidly at the tenth day after birth Radiothyroidectomy at birth led to a significant decrease in succinic dehydrogenase activity (it had little effect on the other enzymes) The activity of succinic dehydrogenase could be restored by thyroid hormone therapy but only if this was begun by the *10th* day after birth if therapy was delayed until the *15th* or 20th day it was without effect The irreversible change which occurs in the brain in the absence of thyroid hormone in the critical period around the 10th postnatal day is illustrated in Fig 14

Rossiter[1386] and Barker[106] failed to observe any changes in succinoxidase activity of brain from thyroidectomized rats treated with thyroid hormone This is probably due to the fact that therapy was started too late after the critical period in brain development had passed

Together with the maturation defects caused by a lack of thyroid hormone behavioural changes have also been recorded in experimental

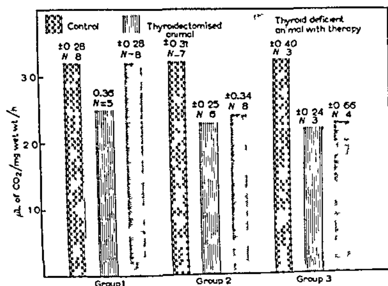


Fig 14

Effect of thyroid replacement therapy on succinic dehydrogenase activity of the cerebral cortex of rats thyroidectomized at birth The success of replacement therapy in restoring enzymic activity to normal values is dependent upon the time when it is begun after thyroidectomy Group 1 replacement therapy started on the 10th post thyroidectomy day and continued for 15 days Group 2 therapy started on 15th day after thyroidectomy and continued for 15–60 days Group 3 therapy started on 20th day and continued for 15 days The standard error of the mean is shown over each bar as also N = the number of animals in each group (From Hamburg and Flexner)

cretinism Eayrs and Taylor[419] have found that changes in cortical structure are associated with delay in the appearance of a number of automatic reactions It was also shown that maze performance aptitude was reduced in cretinous rats this could be improved by thyroid feeding even when it was started as late as the 24th day after thyroidectomy[416, 418] In this connection it must be noted that although defects in behaviour are irremediable in most human cretins Tredgold and Tredgold[1631] have succeeded in improving mental status of these patients if thyroid therapy was begun within the first year of life More recently Hamburg and Vicari[668] have described experiments in which administration of triiodothyronine to new born rats advanced the appearance of eye opening ear unfolding response to audiogenic seizures and swimming reflex by between 1 and 4 days they conclude that thyroid hormone acts as a trigger activating various components of the central nervous system

The ten cycle alpha wave rhythm observed during electroencephalographic (EEG) studies on the posterior portion of the skull was first recorded by Berger[150] Rubin *et al* [1395] showed that both hyperthermia

and thyroxine administration increased the number of alpha waves per second Lindsley and Rubinstein[965] showed that there was a high coefficient of correlation between the alpha rate and total caloric output, but that the coefficient of correlation between alpha rate and BMR was low Ross and Schwab[1383] also found a poor correlation between the alpha rate and BMR the correlation was good when the patient was well fed and mentally relaxed rather than starved and basal Bertrand *et al*[161] reported profound disturbances in the alpha rhythm in hypothyroidism these were more marked in congenital hypothyroidism than in myxoedema appearing in later life in some cretins the alpha wave was absent and when present was found to be greatly retarded Similar findings have been described by Vague *et al*[1660] These changes in alpha waves can be corrected to some extent by thyroid therapy[1419]

Abnormal EEG patterns have also been recorded in hyperthyroidism[302, 303, 1478] Skanse and Nyman[1478] believe that thyrotoxicosis in their patients is the cause of major convulsive seizures and suggest that it can increase the frequency of attacks in epileptics Thyrotoxicosis also produces gross neurological disturbances such as coma, bulbar palsies and athetotic and choreiform movements[1689] In experimental animals daily injection of small doses of thyroxine to rats lowered the convulsive threshold and prolonged the seizures injection of thyrotrophin produced cortical hyperexcitability[1 00] Although similar results were not obtained in the dog it has been shown that the excitatory and inhibitory processes of the cerebral cortex are more sensitive to a small excess of thyroid hormone than is the BMR[96] Administration of thyroid hormones also increases brain excitability in rats as measured by the electroshock threshold and slightly accelerates recovery from electroshock seizures[1615] At the same time changes in electrolyte distribution occur resulting in a decreased extracellular sodium and increased sodium space it is worth noting that in these experiments the relative stimulatory potencies of triiodothyronine and thyroxine were 5 to 1

A relationship has also been established between cerebral oxygen consumption blood flow and thyroid activity Himwich and co workers have found that in human cretins the cerebral arteriovenous oxygen is 5 74 volumes per cent compared with 7 volumes per cent in normal subjects[738] This difference is greater than at first appears because of the slower blood flow in the brain of cretins Although the cretinous brain receives a fairly adequate supply of oxygen it uses less than the normal brain In myxoedema also cerebral blood flow is decreased and cerebral vascular resistance increased[1419, 1447] Sensenbach *et al*[1447] failed to observe the simultaneous decrease in oxygen and glucose consumption reported by Scheinberg and co workers[1419] On the other hand increased cerebral blood flow and decreased cerebral vascular resistance have been reported in

hyperthyroidism[1447, 1503] although cerebral oxygen and glucose consumption remained unaltered[1419, 1503, 1447]

Most workers agree that the oxygen consumption of brain tissue excised from adult animals shows no response to previously administered thyroid hormone This distinguishes the brain from tissues such as liver kidney *spleen heart skeletal* muscle and salivary gland Similarly an absence of effect on brain tissue has been observed after thyroidectomy or thiouracil *treatment*[1031, 602, 5, 114, 116] *see also* [103] *The explanation for the failure* of brain tissue to respond to changes in thyroid status may be found in the work of Reiss *et al* [1290] these authors have shown that thyroxine triiodothyronine and thyrotrophin all raised oxygen consumption in the brains of new born rats and suggest that the action of the thyroid hormones on brain in new born animals can occur because at this time the blood brain barrier for these hormones is still undeveloped Tata (unpublished) has demonstrated a marked fall in the permeability of rats brain to ^{131}I labelled thyroxine and triiodothyronine between the 6th and *21st postnatal* days

The relationship between thyroid hormone function and mental disease is still incompletely *understood In 1888 a Special Report of the Com*mittee of the Clinical Society of London on Myxoedema described myxoedematous patients in whom signs of imperfection of mental processes could be found in advanced cases acute or chronic mania dementia and melancholia supervened Asher[56] has noted that sometimes psychoses occur in myxoedematous patients Miller[1086] has been able to rehabilitate psychiatric patients with myxoedema after they were restored to a euthyroid state Thyrotoxicosis has also been found to result in mental agitation and even in manic psychoses[409, 806]

Thyroid dy*sfunction has been noted in* schizophrenic subjects[901, 09, 767, 768, 1774, 218, 1 9, 1291, 3 9] (see [1409]) and schizophrenia has been reported to be associated with hypo and hyper thy*roidism* The more recent work however demonstrates that schizophrenics as a group have no particular thyroid abnormality

We conclude that the thyroid hormone is essential during maturation of the central nervous system its effect on the proper functioning of the brain and its association with any particular mental disorder have not yet been conclusively established although a possible relationship between hyperthyroidism and Parkinson s *disease has recently been shown*[124]

(VI) Effect on Lactation

The thyroid hormone is not essential for the development of the mammary gland or for the induction of lactation but it is now recognized as an important hormonal factor in the regulation of lactation The report of

Hertoghe[730] that administration of thyroid stimulated lactation in cows remained almost unnoticed for nearly 40 years when Graham[607, 608] described this effect once again and in greater detail He showed that thyroidectomy depressed *milk yield while feeding of dried thyroid* or thyroxine increased not only the amount of milk secreted but also its fat content This work was soon confirmed by Jack and Bechdel[804] Jones[8 1], and Folley and White[507] Since then the powerful but temporary galactopoietic action of thyroid hormones has been exhaustively investigated in humans[1331, 1376, 1336] dairy animals[508, 509, 1264, 1490, 1 86, 174, 175, 176, 49, 736, 83, 933, 1 7, 1640] and in other laboratory animals[501, 506, 833, 3 3, 1256] In the cat, the need for thyroid hormone at the onset of lactation appears to be so great that within a few days there is an almost total disappearance of the colloid in the thyroid gland which shows normal activity during gestation[1256] Singh *et al*[1474] have shown that higher thyroid activity in ewes led to a greater weight gain in lambs because of increased milk yields The whole subject of the thyroid hormone and galactopoiesis has been adequately reviewed in recent years (see [1 84, 505, 177, 502])

The commercial interest in the use of the thyroid hormones for stimulation of galactopoiesis in dairy animals has been largely responsible for these extensive studies Most of the work has involved the use of the artificially iodinated proteins (principally iodocasein) since they are cheap to prepare The galactopoietic potency of the iodoproteins is due to their thyroxine content[177] and a direct relationship exists between the amount of iodoprotein fed and the increase in milk yield The use of iodoproteins has certain disadvantages among them are the need for constant biological standardization of the preparations and the large amounts of non thyroxine iodine ingested by the animal For these reasons the administration of thyroxine is to be preferred Fig 15 illustrates the effect of feeding different amounts of synthetic L thyroxine on the milk yield in cows Notice the sharp decline in the increased milk secretion once the thyroid hormone was withdrawn Later Bartlett *et al*[1 7] used triiodothyronine in similar experiments and found that this compound had only slight galactopoietic activity when given by mouth They believe that the rapid inactivation of triiodothyronine is due to micro organisms in the rumen since subcutaneous administration of triiodothyronine increased its galactopoietic activity

A change in composition of the milk accompanies the increased yield after thyroid hormone administration The most notable change is that the fat content is consistently increased[507, 174, 1671, 1 7] (see [177]) In some cases the increase in fat content was proportionally higher than the increase in milk yield[1282, 1671] low graded doses of thyroid hormone increased only the lipid content *without affecting the amount of milk secreted*[1278] Smith and Dastur[1490] observed similar effects of thyroxine on both milk and

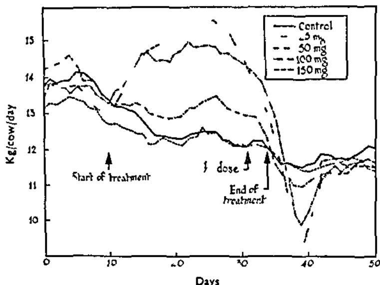


Fig 15

Effect of oral L thyroxine on the milk yield of cows (From Folley[50])

plasma lipids Among other changes produced in milk composition are an increase in the lactose content[507 1490 73] and a decrease in vitamin C content[274] most mineral constituents except for phosphorus and nitrogen content are unaltered[177] The considerable decrease in phosphatase titre of milk after thyroid hormone administration first observed by Folley and White has been confirmed by many workers Concomitantly with the decrease in phosphatase activity the amount of phosphorylated thiamine (vitamin B_1) or co carboxylase increases with respect to total thiamine [84 274 73] Folley[50] has suggested that free or unphosphorylated thiamine in milk results from dephosphorylation of thiamine phosphate while the milk is in the udder This hypothesis awaits confirmation

The iodine content of milk is increased by administration of iodocasein and iodoardein [175 1 8] but most of it can be accounted for as iodide derived from deiodination of the iodoprotein[320] Thyroxine has never been unequivocally demonstrated in the milk[1 87 34] and the mammary gland has been shown to be impermeable to thyroxine[1099 777]

Among factors that influence the galactopoietic action of thyroid hormones are species differences These factors have been studied in ruminants principally the cow[177] the response to thyroxine is maximal during mid lactation in cows[727] but even at this time different breeds of cows vary in their milk yield and their response to thyroxine Thomas *et al*[1605] conclude that it is not possible to make a good cow from a poor cow by administering thyroprotein

So far the mechanism of galactopoietic action of thyroid hormones has not been elucidated. The stimulation of milk secretion probably reflects general stimulation of metabolic activity in the whole animal. There is general agreement with the hypothesis proposed by Folley and Malpress[505] namely that the most likely role of the thyroid hormone in galactopoiesis is to stimulate the circulation, thus providing a richer supply of milk precursors, and further to stimulate the metabolic rate of the alveolar cells themselves. In this connection Folley and French[503, 504] have shown that the metabolic rate of mammary tissue is correlated with the onset, development and decline of lactation.

(VII) Effect on the Cardiovascular System and Blood

Tachycardia and an increased pulse rate are symptoms characteristic of hyperthyroidism. Crooks and Murray[338] have shown that a sleeping pulse rate of over eighty in the human is probably diagnostic of a hyperthyroid condition. Cardiac output and pulmonary ventilation are also increased in hyperthyroidism and decreased in thyroid deficiency[550, 1144, 1419, 8]. It appears therefore that while the thyroid hormone increases the consumption of oxygen in peripheral tissues, it also provides the means of satisfying the increased demand for oxygen.

The action of the thyroid hormones on the heart and circulation have been considered as a secondary effect of the overall stimulation of metabolism[1149, 1306, 486]; there is, however, an increasing belief that the calorigenic action of thyroid hormone and its stimulation of the heart are independent[1075, 1269, 1074, 9, 6, 1073]. Similar conclusions were drawn by Hoffmann *et al.*[746] when they found that the heart of the hyperthyroid animal produces more of an adrenaline-like substance than does normal heart or that of a hypothyroid animal. These authors also pointed out that in hyperthyroidism the circulatory system works less efficiently, in that work done is not reflected in a correspondingly increased supply of blood. Raab[1254, 1255] and Gross and Greenberg[630] suggested that cardiac effects in hyperthyroidism were due to the synergistic action of thyroxine and adrenaline. Recently it has been shown[1045] that thyroxine enhances the hypertensive action and vascular damage produced by adrenal steroids; necrosis of the heart and gut following renal injury can be prevented by thyro-parathyroidectomy[936]. The effects of thyroxine and triiodothyronine on oxygen consumption of isolated auricles and ventricles have also been studied[739, 593]; triiodothyronine had a potency ten times greater than that of thyroxine in stimulating rat heart auricle respiration. The rate of blood flow through various organs is not proportional to their oxygen consumption; factors controlling haemodynamic effects of hyperthyroidism have recently been discussed by Thomas[1604].

Besides its action on the cardiovascular apparatus, the thyroid hormone

is thought to effect changes in the composition of blood Since Kocher's[870] first observations there have been many reports associating hypothyroidism with anaemia Stern and Altschule[1540] showed that the haemoglobin level fell in patients who had undergone thyroidectomy for heart disease the severity of the anaemia being related to the fall in BMR The anaemia of hypothyroidism has been associated with a decreased activity of the bone marrow[1090, 1545, 1449], it has also been considered as a physiological adaptation to the decreased oxygen requirement[194, 186] Seip[1439] believes that the anaemias of hypothyroidism in infants and adults result from such an adaptive process, and has shown that erythropoiesis is stimulated by thyroxine treatment in both these types of anaemia Adam and Doljanski[9] have shown that radiothyroidectomy in the new born results in a progressive anaemia In an extensive study Larsson[912] has demonstrated different types of anaemia in myxoedema most of his patients responded completely to thyroid therapy alone and he believes that iron therapy is con*traindicated in these subjects until the hypothyroidism has been treated*

In adult anaemias associated with hypothyroidism hyposideraemia is common and in some cases the iron binding capacity of the serum is lowered Austoni *et al*[80] have studied iron uptake (as measured with ^{59}Fe) by bone marrow and blood in rats and have shown that it is decreased and retarded after thyroidectomy The iron content of spleen and liver rises after thyroidectomy this may represent a means of disposal of iron not required when erythropoiesis is depressed

In hyperthyroidism polyerythraemia is usually absent but the bone marrow may be hyperplastic[82] Larizza *et al*[908] found that serum iron levels were depressed in this condition which they suggested resulted from a greater need for iron in the tissues Pernicious anaemia is sometimes found in association with myxoedema whether the thyroid deficiency actually causes this type of anaemia is not known[1065, 1676, 1428, 353, 206]

(VIII) Other Actions of the Thyroid Hormones

Besides the physiological actions of thyroid hormones described above many others have been recorded in the literature These include effects on carbohydrate metabolism vitamin requirements renal function reproduction anaphylactic reactions and wound healing It is beyond the scope of this book to describe these effects in detail many represent secondary effects resulting from alterations in the metabolic rate or from endocrine imbalance in other endocrine systems in hyper and hypo thyroidism

Carbohydrate Metabolism

Early work on the effects of thyroid hormone on carbohydrate metabolism did not yield any startling results Cramer and M Call[328] showed that thyroidectomy was without effect on carbohydrate metabolism in rats

the claim of Althausen and co workers that the thyroid hormone exerts a specific action on the rate of intestinal absorption of certain sugars in the rat[43, 40, 41] and of galactose in man[4, 14, 6] has not been confirmed[1121, 611, 217] Effects of thyroid feeding on glycogen mobilization in rats and dogs[3, 5, 1192] and in other species[890, 1377, 400, 3, 914] has only been demonstrated when large doses of thyroid hormone were given This casts some doubt on a physiological role of the thyroid hormone in the mobilization of liver glycogen

Thyroid status does not appear to have much influence on the course of diabetes[770, 1148] and sugar metabolism is not greatly altered in hyperthyroidism[1410, 1762, 990] It seems that in a hyperthyroid state an existing glycosuria may be somewhat increased[811, 813, 8, 2] but rarely causes glycosuria to appear

Vitamins

The effect of the thyroid gland on the body s vitamin requirement has been studied for many years the requirements and turnover of thiamine[14, 7, 1396] riboflavin[404] nicotinamide[1554] and vitamin B_1[1770, 889], are all increased in hyperthyroidism and decreased in hypothyroidism this subject has been reviewed by Rawson *et al*[1276] It is likely that increased vitamin turnover is secondary to the overall stimulation of metabolism after thyroid therapy such an assumption seems justifiable since most vitamins are now believed to act as co factors for enzymes and enzyme systems which are themselves affected by changes in the metabolic rate

Other Effects

Effects of thyroidectomy and thyroid administration have been studied in connection with reproduction in various species[330, 1593, 1780, 83, 1648, 58, 874, 1679] The relationships of thyroid hormone status with renal function[424, 308, 684, 1189] and with sensitivity to bacterial injections, immunization and anaphylaxis (see [1276] for early references also [1696, 989, 1159]) have also received much attention The specificity of thyroid hormone action on these different functions has not however, been unequivocally demonstrated

(IX) THE LATENT PERIOD OF ACTION OF THYROID HORMONES

The thyroid hormones are remarkable in that they have a latent period of action and it has not yet been explained why an injection of thyroxine in a thyroidectomized animal depleted of thyroid hormone stores should not produce a detectable response for hours or even days In the athyreotic human the first appearance of metabolic or other responses only occurs 2 days after administration of thyroxine The latent period of action is independent of the dose and of the magnitude of the response finally obtained

Two arguments have been advanced in an attempt to explain the latent period of action of thyroid substance and of thyroxine (1) The thyroid

A study of these tables immediately shows that very different potencies for any one compound are obtained depending on the method of assay and on species differences thus the response of amphibia is in general much greater than that of mammals and is also less specific compounds which have little or no activity in mammals (Table III) can stimulate amphibian metamorphosis The response of different species can also be seen to vary in the same test, this is seen in the stimulation of oxygen consumption by O methyl DL thyroxine in man the guinea pig and mouse Further a compound may have different potencies towards oxygen consumption and in suppression of the pituitary this is found for 3 5 3 triiodo L thyronine and 3 5 3 5 tetraiodothyroacrylic acid in the rat

Another factor must be considered when we try to explain differences in potencies determined by any method of assay in the same species namely the method by which the compound has been synthesized Certain preparative procedures may involve contamination of the final product with small amounts of impurities which if they possess high physiological activities themselves will mask the true activity of the compound being assayed Finally, the health of the animal and nutritional and seasonal variations may all influence biological assays of these compounds

TABLE II

Relative potencies of halogated thyronines compared to L thyroxine

Compound	Species	Physiological test		
		Total O_2 consumption	Growth and differentiation	Goitre prevention (or TSH depression)
L Thyroxine	All species*	100	100	100
D Thyroxine	Man	10–16	—	—
	Guinea pig	—	—	30
	Tadpole	—	0	—
3 5 3 Triiodo L thyronine	Man	90–140	—	280–540
	Rat (normal)	100–175	—	250–600
	Rat	100–200	500	—
	Chick	100	—	100
	Tadpole	—	500	—
3 5 3 Triiodo D thyronine	Rat	—	—	14
3 3 5 Triiodo DL thyronine	Rat	—	—	5
	Tadpole	—	0–5	—
3 5 Diiodo L thyronine	Rat	—	—	5
	Tadpole	—	40	—
3 5 Diiodo DL thyronine	Rat	3–5	—	—
	Tadpole	—	2 5	—

*In all cases man represents the myxoedematous subject

Thibault[1600] that the thyropyruvic acids had latent periods intermediate between those of the amino and acetic acids gave support to the idea that the thyroacetic acids represented the active forms of the thyroid hormones this work however has not been confirmed[115 377 1733] There are moreover further objections to this hypothesis (1) The latent periods of action of triiodothyronine and triiodothyroacetic acid are the same in the myxoedematous subject[1733] (2) If the acetic acids were 'true' hormones formed in the cells then their transfer *inwards* across the cell membrane which would have to follow injection into the animal or addition to incubation media *in vitro* would represent an unnatural process (3) A rapid conversion of thyroxine and triiodothyronine to the thyroacetic acids has been observed both *in vivo* and *in vitro* the time taken for this conversion is very much shorter than is the latent period of action of either of the corresponding amino acids These observations preclude the possibility that the acetic acids are the active compounds derived from the circulating hormones

From the above considerations there is no evidence to support the hypothesis that the hormonal secretions of the thyroid gland are converted to active compounds in the tissues either by partial deiodination or by modification of the side chain It may be that the latent period of action of thyroid hormones is concerned with their steady distribution to the peripheral targets and that a search for derivatives without latent periods has led us in the wrong direction in the elucidation of thyroid hormone action

(X) Chemical Structure and Biological Activity of Substances Related to Thyroid Hormones

In recent years many attempts have been made to correlate the physiological activities of compounds related to thyroxine with their chemical structure[554 692 540 103 105 1 4 1441 41 10 9 1133 1081] As a result of all this work a few generalizations can now be made but before considering these a number of discrepancies in the findings of different workers must be discussed

In Tables II III and IV are shown the potencies of a number of compounds structurally related to thyroxine the method of assay and the species in which the assay has been made These include total oxygen consumption growth and differentiation and prevention of thiouracil induced goitre Total oxygen consumption represents the total increment obtained during the period of stimulation and not the rate of stimulation in order to exclude any effects on speed of action the mouse anoxia test has been excluded from these tables The data included are taken from the above and following publications [1288 1452 1455 1638 1640 953 300 1365 57 1563 955 956 169 1389 1390 1391 1598 1600 606 408 1151 109 1233 1442 1 34 1733 1097 1332 1458 1530]

TABLE III

Relative potencies of derivatives of iodothyronines modified in the alanyl side chain

Compound	Species	Physiological test		
		Total O_2 consumption	Growth and differentiation	Goitre prevention (or TSH depression)
L Thyroxine	All species	100	100	100
3 5 3 5 Tetraiodothyrobutyric acid	Tadpole	—	1200	—
3 5 3 Triiodothyrobutyric acid	Tadpole	—	1200	—
3 5 Diiodothyrobutyric acid	Tadpole	—	300	—
3 5 3 5 Tetraiodothyropropionic acid	Rat	30–60	—	20–38
	Tadpole	—	5 000–10 000	—
3 5 3 Triiodothyropropionic acid	Rat	—	—	50
	Tadpole	—	13 500–30 000	—
3 3 5 Triiodothyropropionic acid	Tadpole	—	12	—
3 5 Diiodothyropropionic acid	Rat	1 0	—	—
	Tadpole	—	600	—
3 3 Diiodothyropropionic acid	Tadpole	—	600	—
3 Iodothyropropionic acid	Tadpole	—	0	—
3 5 3 5 Tetraiodothyropyruvic acid	Rat	10–12	—	—
	Tadpole	—	15	—
3 5 3 Triiodothyropyruvic acid	Tadpole	—	50	—
3 5 3 5 Tetraiodothyroacrylic acid	Rat	1 5	—	20–25
	Tadpole	—	150–1000	—
3 5 3 Triiodothyroacrylic acid	Tadpole	—	3300	—
3 5 Diiodothyroacrylic acid	Rat	0 1–0 2	—	—
	Tadpole	—	24	—
3 5 3 5 Tetraiodothyroacetic acid	Man	0–5	—	—
	Rat	100	—	50
	Tadpole	—	500–1000	—
3 5 3 Triiodothyroacetic acid	Man	0–15	—	—
	Rat	125	—	50 150
	Tadpole	—	1000–2400	—
3 5 Diiodothyroacetic acid	Tadpole	—	30	—

TABLE II (cont)

Compound	Species	Physiological test		
		Total O_2 consumption	Growth and differentiation	Goitre prevention (or TSH depression)
3 5 Diiodo DL thyronine	Man	0–10	—	—
	Tadpole	—	0	—
3 3 Diiodo DL thyronine	Rat	0–10	—	0 6 82
	Tadpole	—	25–35	—
2 6 Diiodo DL thyronine	*Man Rat*	0	—	0
3 Iodo DL thyronine	Tadpole	—	0	—
3 Iodo DL thyronine	Tadpole	—	0	—
3 5 3 5 Tetrabromo DL thyronine	Man	0–5	—	—
	Rat	0–3	—	5
	Tadpole	—	0–10	—
3 5 3 Tribromo DL thyronine	Man	80–100	—	—
	Rat	—	—	17–22
	Tadpole	—	28	—
3 5 3 5 Tetrachloro DL thyronine	Man	0–0 2	—	—
	Rat	—	—	0–0 2
	Tadpole	—	2–12	—
3 5 3 Trichloro DL thyronine	Rat	—	—	0 5
3 5 Dichloro DL thyronine	Rat	0–0 5	—	—
3 Fluoro DL thyronine	Rat	—	—	0
3 5 Diiodo 3 5 dibromo DL thyronine	Rat	0 5	—	3 6
	Tadpole	—	>10	—
3 5 Diiodo 3 bromo DL thyronine	Rat	—	—	70–100
3 5 Dibromo 3 5 diiodo DL thyronine	Rat	5–8	—	6
	Tadpole	—	0–5	—
3 5 Dibromo 3 iodo DL thyronine	Rat	—	—	65
3 3 Diiodo 5 bromo DL thyronine	Rat	*50–70*	—	50–70
3 5 Diiodo 3 5 dichloro DL thyronine	Rat	0 4	—	22
	Tadpole	—	48	—
3 5 Diiodo 3 chloro DL thyronine	Rat	—	—	4
	Tadpole	—	18	—
3 5 Dichloro 3 5 diiodo DL thyronine	Rat	0–0 1	—	—
3 5 Diiodo 3 5 difluoro DL thyronine	Rat	—	—	0 75–2
3 5 Diiodo 3 fluoro DL thyronine	Rat	—	—	1 2–5
3 5 3 Triiodo 5 fluoro DL thyronine	Rat	—	—	17

TABLE IV (cont)

Compound	Species	Physiological test		
		Total O_2 consumption	Growth and differentiation	Goitre prevention (or TSH depression
3 5 Dinitro DL thyronine	Rat	—	—	0
3 5 Dinitro 3 5 -diiodo DL thyronine	Rat	—	—	0
3 5 Diiodo 3 5 dinitro DL thyronine	Rat	0	—	—
Sulphur analogue of DL thyroxine	Man	5–12	—	—
	Rat	—	—	0 5
	Tadpole	—	8 5	—
Sulphur analogue of 3 5 3 triiodo DL thyronine	Rat	—	—	73
	Tadpole	—	95	—
3 5 Diiodo 3 5 dimethyl L thyronine	Tadpole	—	140	—
DL Thyronine	Man	0	—	—
	Rat	0	—	0
	Tadpole	—	0	—

The following conclusions concerning the relationship between physiological activity and chemical structure can be reached from the data presented here and from the references cited above (1) The diphenyl ether grouping i essential for activity (the iodotyrosines are biologically inert) (2) Thyronine derivatives must contain at least two halogen substituents in order to exert calorigenic or pituitary depressing effect (3) Substitution with iodine yields compounds with the highest potency substitution with bromine and chlorine yields compounds with progressively decreasing potency (4) Trihalogenated compounds substituted in the 3 5 and 3 positions are more active than the corresponding tetrahalogenated compounds *Only two exceptions to this rule have so far been recorded* 1133 1455 (5) The position of the substituents is important thus *ortho* - and *meta* thyroxine have virtually no activity when compared with the naturally occurring isomer nor has 2 6 diiodothyronine (6) Variations in the side chain of the thyroxine molecule produce a marked effect on physiological activity this is shown in the series where the alanine side-chain is substituted by aliphatic acids optimal activity is manifested by the derivative with the 3 carbon residue i e the thyropropionic acid A decrease in the length of the side chain to the thyroacetic acid or thyroformic acid produces a decrease in activity an increase in the length of

TABLE III (cont)

Compound	Species	Physiological test		
		Total O_2 consumption	Growth and differentiation	Goitre prevention (or TSH depression)
3 5 3 5 Tetraiodothyroformic acid	Rat	—	—	<0 1
	Tadpole	—	7–25	—
3 5 3 Triiodothyroformic acid	Tadpole	—	60–200	—
3 5 Diiodothyroformic acid	Tadpole	—	30	—
Glycine analogue of thyroxine	Rat	—	—	0 16
	Tadpole	—	35	—
Thyroxamine	Rat	0–50	—	—
	Tadpole	—	0–50	—
3 5 Diiodothyronamine	Mouse	0	—	—
	Tadpole	—	0	—

TABLE IV

Relative potencies of other derivatives of thyroxine

Compound	Species	Physiological test		
		Total O_2 consumption	Growth and differentiation	Goitre prevention (or TSH depression)
L Thyroxine	All species	100	100	100
DL *Ortho* thyroxine	Rat	1–2	—	—
DL *Meta* thyroxine	Rat	0	—	—
O Methyl DL thyroxine	Man	0–3	—	—
	Guinea pig	>50	—	—
	Mouse	25	—	—
	Tadpole	—	50	—
N Acetyl DL thyroxine	Man	12–17	—	—
	Rat	5	—	11
	Tadpole	—	1–1 5	—
DL Thyroxine methyl ester	Mouse	33	—	—
DL Thyroxine ethyl ester	Mouse	25	—	—
3 5 3 5 Tetranitro DL thyronine	Rat	?	0 5	—
	Tadpole	—	0 05	—

CHAPTER 6

SOME CURRENT CONCEPTS OF THE MECHANISM OF ACTION OF THYROID HORMONES

THE major physiological actions of the thyroid hormones are now well established but the mechanism involved in eliciting these actions is still unknown. This deficiency in our knowledge is recognized by many physiologists and biochemists at the present time and is evident from the vast and rapidly increasing literature that has now accumulated on this problem.

The question of how the thyroid hormones act on their target cells has interested investigators ever since the first reports on the properties of thyroid secretions began to appear at the end of last century. The manner in which the answers have been sought has however greatly changed and has evolved as a result of advances made in biological and physical sciences during the last sixty years. As with the attempted explanation of many biological phenomena this problem was first attacked at the level of the whole organism or a particular organ and has passed to the tissue, the cell and subcellular structures, more recently, with the intervention of biochemists and biophysicists, it has been extended from the molecular to atomic and subatomic levels. Unfortunately, as Levine[259] has pointed out, the concept of the *mode* of action of hormones has often been confused with their "effect", "function" or "action", and many hypotheses of the mechanism of thyroid hormonal action merely elaborate the effect at one of the above levels rather than telling us how the action itself is brought about. Furthermore many investigators have accepted the idea that the variety of physiological actions of thyroid hormones are manifestations of a single action—that of control of metabolic rate; this has led to an accumulation of information on possible mechanisms involved in the stimulation of metabolism, while the questions of the mechanism of thyroidal control of growth and metamorphosis and the effect on body fluids in myxoedema has almost been ignored. One of the reasons for this one-sided view of the problem is that ideal methods for studying the mode of action of thyroid hormones have not yet been developed; nevertheless the knowledge gained during the last decade is impressive and in itself warrants a brief discussion. For more detailed discussions of both the mechanism of hormone

the side chain (as in the thyrobutyric acids) again decreases activity Further, the glycine analogue of thyroxine is less active than thyroxine (7) Compounds in which the carboxyl group of the side chain is absent (the iodothyronamines) or blocked (thyroxine esters) exhibit diminished potency (8) The degree of ionization of the phenolic group may be important If we take triiodothyronine as the most active compound in the thyronine series thyroxine in which the phenolic group is more highly dissociated is less active 3 5 diiodo 3 5 dinitrothyronine, with an even more acidic phenolic group possesses no activity in the rat Depression of ionization of the phenol group (3 5 diiodothyronine, 3 5 diiodo 3 5 dimethylthyronine) also results in decreased activity

From these considerations we can postulate the following broad generalizations Substitution of the diphenyl ether residue with halogen atoms gives compounds with decreasing potency when the substituents are iodine, bromine and chlorine in that order The optimal length for the side chain in a given series is a 3 carbon residue The physiological significance of these structural requirements remains to be elucidated

mechanism of action of the hormone Some workers have even suggested a direct interaction between the thyroid hormone and vitamins but none of these suggestions has obtained any degree of acceptance Sadhu and Brody[1403] explained the antagonism between vitamin A and thyroxine on the basis of a transfer of iodine atoms from the hormone to the vitamin thus thyroxine would be inactivated and the iodinated vitamin would at the same time be capable of suppressing the pituitary secretion of TSH A large section of opinion is at the present time in favour of a direct action of thyroid hormones on enzymes and any interaction between vitamins and these hormones has become of minor importance

Among the earlier hypotheses of the mode of action of thyroid hormones one that is still acceptable but does not explain many of the physiological properties of thyroid hormones is that of a permissive action of one endocrine secretion on another or of a potentiation effect We have seen examples of this in Chapter 5 in the synergism between growth hormone and thyroxine on body growth and bone development and between the corticosteroids and adrenaline and thyroxine on basal metabolism or response to cold[1094, 433] Attempts to understand the mechanism of action of thyroid hormones by studying their interrelationships with other hormones introduce many complications in interpretation since the mode of action of any hormone is not yet clearly understood

(II) Current Hypotheses

(a) *Direct Action on Enzymes*

In 1941 Green[612] in his general consideration of Enzymes and Trace Substances put forward the theory that hormones in common with other trace substances in the cell such as vitamins metal ions and drugs produce their biological effects either by participating in enzyme systems as co enzymes or by specifically inhibiting key enzymes There was a sound basis for assigning a function to vitamins (especially vitamins B) as co enzymes or for some drugs (such as eserine ephedrine and sulphanilamide) as specific enzyme inhibitors but similar direct evidence was lacking to explain the mechanism of hormonal action However this theory was immediately accepted as explaining the action of most hormones including thyroxine further no other satisfactory explanation could be given for the earlier observations on the responses of isolated tissues from animals made hypo or hyper thyroid or for the interaction between thyroid hormone and certain vitamins

Prominent among the earlier work are the observations of Rohrer[13, 3] and Foster[518] of increased oxygen consumption in isolated tissues from mice fed on desiccated thyroid and decreased oxygen consumption of tissues from thyroidectomized rats Since this work it has been repeatedly

action in general and more specifically that of thyroid hormones the reader is referred to the following works[612 613 103 411 1039 905 16 902 903 959]

(I) Earlier Theories

Among the earlier suggestions acceptable to many biologists on the mode of action of thyroid hormone was that of an innervation of the target organs The evidence presented was to a large extent indirect, was without sufficient controls and was difficult to reproduce Thus on the basis of experiments performed on animals with lesions of the nervous system and the failure of thyroid extracts to produce an increase in oxygen consumption of tissues *in vivo* it was concluded either that innervation was essential for thyroid hormone action or that the thyroid gland made the target cells more responsive to the sympathetic nervous system[78 1189 1307 1076 245 43] Negative evidence was however produced to demonstrate the fallacy of this hypothesis such as the failure of denervation to affect the response of various organs to thyroxine or alter metabolic responses in sympathectomized animals[1060 1310 1 45 1033 10 7 1556] Later the work of Mansfeld and his colleagues disproved even more conclusively the early suggestions that the effects of the thyroid were mediated through the central nervous system nevertheless these authors believed that the nerve trunks leading to the target organs were involved in some way in thyroid hormone action[10 2 10 0 10 1] At the present time there is general agreement that innervation of the target organs does not represent a mechanism of action of the thyroid hormones although this hypothesis is still found in current Russian literature[1172 800 1469] It should be emphasized that whereas thyroid hormones have a very definite and profound effect on the central nervous system (Chapter 5) it is doubtful whether physiological effects such as regulation of metabolism or protein synthesis and breakdown are dependent on the mediation of the higher nervous activity

The discovery of vitamins and their importance in the regulation of a wide variety of biological processes was soon followed by a tremendous amount of work too voluminous to be cited here on the relationship between thyroid function and vitamin requirement or metabolism This work has been reviewed up to 1943 by Drill[403] and more recently by Rawson *et al*[1 76] Whereas most authors have merely described the effects of a deficiency or excess of vitamins on the thyroid gland or symptoms of vitamin deficiency caused by an excess of thyroid hormone some have concluded that the vitamin balance is intimately involved in the mechanism of hormone action The most common effect produced by the administration of thyroid hormone is an increased need for vitamins A B_1 B_1 and C which *eventually leads to deficiency symptoms It is likely that* the increased requirement is due to the overall acceleration of most metabolic processes, but this does not provide us with a means of understanding the

mechanism of action of the hormone Some workers have even suggested a direct interaction between the thyroid hormone and vitamins but none of these suggestions has obtained any degree of acceptance Sadhu and Brody[1403] explained the antagonism between vitamin A and thyroxine on the basis of a transfer of iodine atoms from the hormone to the vitamin thus thyroxine would be inactivated and the iodinated vitamin would at the same time be capable of suppressing the pituitary secretion of TSH A large section of opinion is at the present time in favour of a direct action of thyroid hormones on enzymes and any interaction between vitamins and these hormones has become of minor importance

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TABLE V (cont)

Enzyme or enzyme system	Effect on activity	References
(14) Acid phosphatase	(c) Hypothyroidism decreases and hyperthyroidism increases renal enzyme	1067
(15) Alkaline phosphatase	(a) Thyrotoxicosis increases bone intestinal skin serum enzyme	1723 135 1240 104 888
	(b) Thyrotoxicosis decreases liver and renal enzyme	889 1047
	(c) Hypothyroidism increases liver and spleen enzyme	883
	(d) Hypothyroidism decreases renal enzyme	885
(16) Glucose 6 phosphatase	Increase in liver enzyme in thyro toxicosis	2012
(17) D Amino acid oxidase	Increase in thyrotoxicosis De crease after thyroidectomy	858 1385
(18) Tyrosine oxidase	Thyrotoxicosis inhibits thyroid ectomy restores activity	97
(19) Tyramine oxidase	Increase in thyrotoxicosis	1710
(20) DOPA decarboxylase	Decrease in thyrotoxicosis	1710
(21) Betaine homocysteine transmethylase	Decrease in thyrotoxicosis	7 3
(22) Alanine glutamic trans aminase	Decrease in thyrotoxicosis	764

TABLE VI

Effect of direct interaction between certain enzymes and thyroid hormones *in vitro*

Enzyme or enzyme system	Effect on activity	References
(1) Succinoxidase	(a) No effect on liver preparations	1726 1727
	(b) Inhibition of liver preparation	1068
	(c) Stimulation of heart liver and kidney mitochondria	36 568 see text
(2) Succinic dehydrogenase	(a) Diphasic effect inhibition above 1 4 $\times 10^{-}$ M and stimula tion below 7 $\times 10^{-}$ M of thyrox ine	15 1 1
	(b) Protection by thyroxine of SH *groups from oxidation*	678
(3) Malic dehydrogenase	Inhibition by thyroxine and ana logues	1 1 1 47 1 0

TABLE VI (cont)

Enzyme or enzyme system	Effect on activity	References
(4) Lactic dehydrogenase	Inhibition by thyroxine and analogues	1146 174
(5) L Glutamic dehydrogenase	(a) Inhibition by thyroxine and analogues	1767
	(b) Inhibition of both forward and reverse reactions	26
(6) Ascorbic acid oxidase	Stimulation at low concentrations of thyroxine and triiodothyronine inhibition at high concentrations	566 4 7 53
(7) Oxidative phosphorylation	(a) No effect	2 1156
	(b) Uncouples phosphorylation from oxidation inhibits phosphorylation with little effect on O_2 consumption	2 4 1 see text
	(c) Increase in P/O ratio with increases in O_2 consumption under some conditions	223
(8) Adenosine triphosphatase	(a) No effect on fresh mitochondria activation in pre aged mitochondria	9 7 0 see text
	(b) Inhibition of myosin ATPase	877
	(c) No effect on activity in digitonin extracts of mitochondria	
(9) Creatinephosphokinase	Inhibition of partially purified and crystalline enzyme	
(10) D Amino acid oxidase	Competitive inhibition	285
(11) Acetyl phosphatase	Inhibition	8
(12) Tyrosine oxidase tyrosine glutamic acid transaminase	Non competitive inhibition	97 78
(13) TPNH DPN transhydrogenase	Inhibition	

The data presented in these tables in no way indicate the nature of a direct intervention of thyroid hormones on enzymic activity Furthermore much of the information is contradictory and there is an impressive lack of uniformity in the experimental methods used However most biochemists and endocrinologists have concentrated their efforts on those enzymes and enzyme systems that play a vital role in the fundamental reactions on which depend the respiratory activity of the cell and the release and utilization of energy provided by foodstuffs The action of thyroid hormones on these enzymes is discussed below

(i) *Oxidation of succinate*—Oxidation of succinic acid to fumaric acid is one of the key catalytic functions of respiratory enzymes, this is ensured by the succinoxidase system which represents the sum total of the activity of succinic dehydrogenase, cytochrome *c* cytochrome oxidase and electron carriers whose nature is largely unknown Much of the work done on the effect of thyroid hormone is based on the response of succinoxidase activity as a whole although more recent investigations have taken into account the effect on the individual components in this system

Dye and Maughan observed in 1929[412, 413] that thyroidectomy in dogs was followed by a 16% decrease in the succinoxidase activity of skeletal muscle Since then it has been shown by various workers that the opposite effect is obtained by administration of desiccated thyroid or thyroxine [1617, 1618, 1068, 1726, 1, 2, 1616, 1495] Barker[106] in a very thorough study, showed that the influence of thyroxine and of thyroidectomy on tissue metabolism in the rat could be directly correlated with the effect on the succinoxidase activity of most tissues he also showed that thyroxine administration reversed the effect of thyroidectomy and that tissues such as brain testis spleen and thymus whose respiratory rate *in vitro* is independent of the thyroid status of the animal, were also unaffected as far as succinoxidase activity was concerned This last effect was briefly described somewhat earlier[1385, 116] Maley[101] has also found an increased succinoxidase activity in the mitochondria isolated from livers of hyper thyroid rats

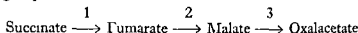
As regards the influence of experimental hypo and hyper thyroidism on the individual components of the succinoxidase system it is now known that thyroidectomy and thiouracil treatment will lower whereas administration of thyroxine desiccated thyroid or TSH will raise the level of cytochrome oxidase (also termed indophenol oxidase) in slices or homogenates of liver heart kidney and skeletal muscle and in liver mitochondria[414, 1031, 1617, 1618, 887, 1012] Tissieres[1620, 1621] found that the amount of cytochrome *c* in rat skeletal muscle decreased by 30% after thyroidectomy or thiouracil administration whereas thyroxine administration increased it to a significant extent in normal and thyroidectomized rats Later, Drabkin[396] performed direct spectrophotometric analysis of total cytochrome *c* in various tissues and obtained similar results he concluded that a possible mode of action of thyroid hormone in regulating oxygen consumption was effected through the control of tissue levels of cytochrome *c* This hypothesis was not accepted and later Drabkin[397] modified it by stating that the change in cytochrome *c* level was not a primary action of thyroid hormone but resulted from some other action Succinic dehydrogenase activity has also been measured independently in liver and thyroxine administration has been shown to result in a higher enzyme activity [103, 13] Aebi and Abelin[13] have suggested that the parallel increase in

BMR, liver succinic dehydrogenase and nitrogen content of the liver after thyroxine administration is due to an increase in the number of mito chondria in liver cells This view has received no support, and in fact Maley (cited in [902]) has found that the increased succinic dehydrogenase activity in hyperthyroid rat livers is accompanied by an increase in mito chondrial nitrogen concentration A point of interest (and one which will be discussed later) is that dinitrophenol which can also stimulate the BMR has no thyroxine like effect on succinoxidase activity or on the total cytochrome *c* content[160, 161, 1495]

As regards the oxidation of succinate the effect of thyroid hormones *in vivo* can be reproduced *in vitro* A brief report by Aloisi and Cavallini[36] that addition of thyroxine to aged heart mitochondrial preparations acti vated succinic oxidase was followed by a detailed description by Gemmill[568] on the stimulation by thyroxine of fresh Keilin Hartree rat heart mito chondrial preparations Although Smith and Williams Ashman[1495] failed to obtain activation in fresh or aged preparations many workers have con firmed the stimulation of the succinoxidase system from various tissues when thyroxine and triiodothyronine are added *in vitro*[1735, 1557, 453, 1550, 1558, 875, 116, 1741, 111] Kripke and Bever[879] have found that the effect of thyroxine is to preserve the succinate cytochrome *c* reductase activity in rat heart homogenates rather than to increase the original activity The inability of Smith and Williams Ashman[1495] to show activation of succin oxidase *in vitro* is probably due to the fact that they used water—rather than phosphate buffer—homogenates Triiodothyronine has an initially more intense but less durable action than thyroxine in stimulating the rat heart system *in vitro* but the overall effect of both compounds is identical on a molar basis[569, 1, 34, 116, 111] In other experiments it was shown that the stimulatory activity of thyroxine *in vitro* was influenced by slight changes in pH[1558] and by the buffer used[568] further the succinoxidase systems of different tissues showed different responses to thyroxine[836, 1551] Sugisawa[1550, 1551] also claimed that thyroxine has a 'diphasic' effect con centrations of thyroxine of about 7×10^{-7}M stimulated while concen trations above 1.4×10^{-6}M inhibited his preparations of mouse and dog liver succinic dehydrogenase

Some suggestions have been made concerning the mechanism by which thyroxine and triiodothyronine stimulate oxidation of succinate Gemmill [568] did not observe a stimulation *in vitro* when methylene blue was used as the electron acceptor and thought that it was unlikely that thyroxine and triiodothyronine acted by removal of an inhibitor of one of the enzymes of the system or by protecting an enzyme from oxidation However, Suzuki[1557] and Sugisawa[1550, 1551] have reported both activation and inhibi tion of succinic dehydrogenase by thyroxine in the presence of cyanide and methylene blue Kripke and Bever[879] suggest that one of the many

possible sites of action of thyroid hormones is at the succinic dehydrogenase level, in which the sulphydryl groups essential for enzymic activity are protected from oxidation other reports point to a non specific effect brought about by the decomposition of an inhibitor or by a limitation of its rate of formation Estabrook *et al*[453] who were able to reproduce the effect of thyroxine on succinoxidase activity by addition of Ca^{++} ions suggested that both substances activate succinoxidase by lowering the accumulation of oxalacetate which is a powerful inhibitor of succinic dehydrogenase Mitochondrial preparations are capable of oxidizing fumarate (the product of oxidation of succinate) to oxalacetate in the following steps



Succinate $\xrightarrow{1}$ Fumarate $\xrightarrow{2}$ Malate $\xrightarrow{3}$ Oxalacetate

Wolff and Ball[1 41] have demonstrated that the addition of thyroxine to rat heart mitochondria causes a considerable diminution in the formation of oxalacetate from malate while it has no effect on the conversion of fumarate to malate They also showed that thyroxine inhibited malic dehydrogenase and did not accelerate the breakdown of oxalacetate Barker[111] has confirmed the inhibition of malate oxidation by thyroxine and a large number of its analogues It is of interest to note that whereas the enzymes catalysing the oxidation of succinate to fumarate are not dependent on pyridine nucleotides malic dehydrogenase is dependent on diphosphopyridine nucleotide (DPN) The direct inhibition by thyroxine and its analogues of isolated malic dehydrogenase which is competitive with DPN has been demonstrated[1746] and the same has been found true for other purified or crystalline dehydrogenases which are DPN linked i e glutamic, lactic, triosephosphate alcohol and glucose 6 phosphate dehydrogenases[1747] *However Barker*[111] *in a recent critical discussion of the significance* of the effect of thyroxine *in vitro* does not think that the stimulatory action of thyroxine on succinoxidase *in vivo* can be accounted for simply by an inhibition of malate oxidation because an unphysiological situation would be created by the accumulation of malate derived from *oxidation of* succinate

(ii) *Uncoupling of oxidative phosphorylation*—So far we have only seen the possible effects of thyroid hormones on enzymic oxidation without considering the energy balance In all biological systems oxidation of foodstuffs is accompanied by the incorporation of a large proportion of the energy thus liberated into high energy phosphate compounds such as adenosine triphosphate (ATP) which in turn are the main source of energy for muscular work protein synthesis and other processes requiring energy The site of coupling of oxidation to phosphorylation is in the mitochondria and depending on the substrate oxidized about two to three

molecules of phosphate are converted to ATP for every atom of oxygen consumed, this is commonly termed the P/O ratio The rate at which oxidative reactions can proceed in mitochondria (and probably in tissues *in vivo*) is controlled by the availability of inorganic phosphate and phosphate acceptors If the formation of ATP from adenosine diphosphate (ADP) is somehow impaired then the energy from oxidative reactions cannot be utilized efficiently and oxidation will continue to proceed at an elevated rate thereby lowering the P/O ratio this is commonly described as uncoupling of oxidative phosphorylation The excess energy in such a situation would be dissipated mostly as heat instead of in the performance of work or anabolic reactions

The possibility that the thyroid hormones controlled the process of oxidative phosphorylation in mitochondria developed from the discovery by Loomis and Lipmann[988] in 1948 that 2 4 dinitrophenol (DNP) possesses the property of uncoupling phosphorylation from respiration DNP can like thyroxine raise the BMR in animals there are however many points of dissimilarity in the effects of the two compounds The first experiments in animals made thyrotoxic with massive doses of thyroxine or desiccated thyroid did in fact show that the phosphorylating efficiency was lowered[1156, 904, 1040] The respiration on the other hand was not markedly altered provided that a phosphate acceptor such as glucose hexokinase was added to the mitochondria In the absence of the phosphate acceptor the respiration of mitochondria from hyperthyroid animals was higher than normal These results although incomplete were interpreted to mean that the thyroid hormone stimulated the BMR by an uncoupling of oxidative phosphorylation which resulted in an increase in oxygen consumption to compensate for the inefficient use of energy released by oxidation It was suggested that the toxic effects of thyroxine on oxidative phosphorylation resulted from the large doses used and represented an extreme condition of the process normally going on in the tissues Among the supporting evidence most often cited for the mechanism of thyroid hormonal action based on a lowering of the P/O ratio is that hyperthyroid animals utilize energy less efficiently than do hypothyroid or normal animals[905, 90, 903] Such a concept would conform with the observations of earlier workers that relatively more net energy is required by a hyperthyroid patient to do the same amount of work than when it is performed by a normal or hypothyroid subject[1, 37, 1467, 40, 1491, 13] Nevertheless clinicians familiar with the measurement of work efficiency in hyperthyroid subjects often point out the serious limitations of the methods used however this may be the theory has been widely accepted and further work has left us in no doubt about the dissociation of phosphorylation from respiration in mitochondria from experimentally hyperthyroid animals[411, 41, 742, 1042, 1015, 1511, 1012, 1013, 1687] The results obtained by

these workers have not always been in agreement because of differences in the methods used namely the degree of thyrotoxicosis, the method of isolating mitochondria, the substrate oxidized and the method of measuring the depression of phosphorylation For example the lowering of P/O ratio in mitochondria from hyperthyroid animals was more marked with succinate and β hydroxybutyrate as substrates than with α ketoglutarate [74, 1015] This suggested that the thyroid hormone might act at a specific step in the oxidation of the substrate or that more than one phosphorylating site was involved, with different susceptibilities to thyroid hormone Attempts to locate any such specific site by varying the terminal electron acceptor in phosphorylation reactions associated with the oxidation of added DPNH have proved unsuccessful[1013]

In spite of a few initial failures[827, 1156] it has now been repeatedly shown that thyroxine triiodothyronine and many related substances will uncouple oxidative phosphorylation when they are added *in vitro* to mitochondria from normal animals[904, 1040, 1041, 4 1, 741, 74, 4 0, 1014, 905, 85, 859, 1123, 1084, 1570, 1568, 1569, 1194, 377, 1013, 1734, 223] Limitations of space prevent us from discussing many interesting aspects of this biochemical property of thyroid hormones in so far as they may explain the mode of action of these hormones some particular points must be mentioned (1) Triiodothyronine is not more active than thyroxine in lowering the P/O ratio [1014, 90, 74, 857] if therefore uncoupling of oxidative phosphorylation *is* assumed to be the mode of action of thyroid hormones then the higher biological activity of triiodothyronine *in vivo* is probably due to more rapid distribution to the tissues (2) The concentration range in which thyroxine and *triiodothyronine act in vitro is very narrow for thyroxine the range* of no uncoupling to that of almost total uncoupling is 5×10^{-5} to 10^{-4} M [1014, 905, 74, 104, 857] This is in contrast to the wide range of concentration at which dinitrophenol is effective[988] (3) In some cases the uncoupling effect is only brought about after a short period (about 30 min) of preincubation of thyroxine or triiodothyronine with the mitochondrial suspension in sucrose solutions at low temperature (0–5 C)[741, 4, 1042, 857, 377, 2 3] Dickens and Salmony[377] found that whereas preliminary contact was necessary for thyroxine to uncouple phosphorylation tri and tetraiodothyroacetic acids caused an immediate and more pronounced uncoupling Bronk[2 3] thinks that the inhibitory effect of thyroxine on phosphorylation after preincubation is due to its combination with a soluble protein released from the mitochondria during preincubation However other workers have obtained the uncoupling effect of thyroxine and triiodothyronine without preincubation especially in saline preparations (4) With regard to the antagonism between certain agents and thyroid hormones on the uncoupling effect the relationship between cysteine and magnesium ions has been *well studied* Cysteine can partially uncouple phosphorylation

from oxidation when glutamate is the substrate but can carry the uncoupling to completion when the oxidation of glutamate is blocked at the succinate level with malonate thyroxine can overcome the uncoupling effect of cysteine in the presence or absence of malonate although the hormone alone has no uncoupling action under the same conditions[471, 905] These authors have suggested that thyroxine antagonizes the effect of cysteine on oxidative phosphorylation by preventing the oxidation of cysteine to cystine Enrichment of the medium with Mg^{+} has been shown to inhibit progressively the uncoupling action of thyroxine and triiodo thyronine and can almost completely restore the phosphorylating ability of mitochondria[85, 1123, 1569, 1734] The possible significance of interaction between thyroxine and metal ions will be discussed later

The effect of thyroid hormones on the breakdown of ATP by mito chondria has been cited as additional evidence for their uncoupling action When mitochondria are extracted in sucrose solution they exhibit little ATPase or apyrase activity this latent activity can be triggered by pre ageing the sucrose suspensions at 37 C for 20 min or by extracting the mitochondria with isotonic KCl[854, 906] Thyroxine triiodothyronine and many related compounds have now been shown to stimulate the release of inorganic phosphate from ATP by pre aged or KCl mito chondria[907, 470, 905, 859, 860, 1012] It has also been shown recently that mitochondria from thyrotoxic rats require shorter pre ageing periods at 37 C than mitochondria from normal animals for the activation of latent ATPase[1012] although an earlier report mentioned increased liver ATPase after administration of thyroxine[1495] Lardy and Maley[905] have reported the effect on mitochondrial breakdown of ATP *in vitro* of twenty com pounds structurally related to thyroxine and triiodothyronine but there is a poor quantitative correlation between biological activity *in vivo* and the action on ATPase *in vitro* for example D thyroxine and the thioether analogue of thyroxine which have little physiological activity stimulate ATP breakdown to about the same extent Dinitrophenol also markedly activates ATPase but the effect of this compound is different from that of thyroid hormones Klemperer[860] has found that at a concentration of 10^{-4} M both thyroxine and DNP activate ATPase in KCl mitochondria at 3×10^{-4} M thyroxine causes an inhibition while DNP continues the activation up to a concentration of 10^{-3} M Another difference between DNP and the thyroid hormones is that the latter will not activate ATPase in mitochondria whose structure has been altered by ageing or by hypo tonic solutions while DNP will Cooper and Lehninger[305] have further shown that both the L and D isomers of thyroxine and triiodothyronine failed to stimulate ATPase in digitonin extracts of rat liver mitochondria while DNP stimulated the enzyme The ATPase activity of myosin is moreover powerfully inhibited by thyroxine, tri and tetra iodothyroacetic

acids[377] Finally, it should be emphasized that the uncoupling of oxidative phosphorylation and ATPase activity may represent two separate and unrelated effects as far as thyroid hormones are concerned, since it has been shown that under certain conditions DNP and cysteine do not activate anaerobic ATPase although they can uncouple oxidative phosphorylation[1739 905]

The question whether thyroxine and triiodothyronine combine with mitochondrial particles before the uncoupling reaction takes place has also been studied[742 90 858] In these experiments mitochondria were kept in contact with ^{131}I labelled hormones and their uptake measured under various experimental conditions including those used for uncoupling studies Mitochondria were found to bind 80–90% of the radioactivity although no marked binding affinity was detected when thyroxine was injected into the intact animal[966 941] In any case, the uptake of thyroid hormones by mitochondria *in vitro* appears to be unrelated to their uncoupling action Hoch and Lipmann[74] observed the same amount of mitochondrial binding of thyroxine and triiodothyronine in experiments in which uncoupling took place (saline preparations) as in experiments in which no uncoupling was observed (sucrose preparations) Further Klemperer[858] has shown that mitochondrial binding of the iodothyronines is the same at 0 C and 20 C and was even higher in heat denatured mito chondria this indicates a passive binding which is independent of metabolic activity

(iii) *Other enzymes*—We have seen in Tables V and VI that the action of thyroid hormones both *in vivo* and *in vitro* is not restricted to those enzymes involved in the oxidation of succinate and malate or to those that take part in the complex sequence of oxidation coupled to phosphorylation Among other enzymes and enzyme systems which have been studied are those that take part in (1) oxidation of other substrates such as lactate pyruvate glutamate and glucose 6 phosphate[168 557 589 1747] (2) hydro lytic processes such as those catalysed by phosphatases and acetylphos phatase[869 883 1047 686] (3) phosphorylating and dephosphorylating pro cesses which involve hexokinase and creatinephosphokinase[1495 83] (4) oxidation and transamination of amino acids[856 764 978 979] Although some of these functions have been implicated in the mode of action of thyroid hormones no coherent theory has been developed as has been done for oxidative phosphorylation However the many difficulties encountered in all attempts to demonstrate thyroid hormonal control of oxidation and phosphorylation may well direct the attention of workers to other enzyme systems Efforts have already been made in this direction[88] in a study of the effect of L thyroxine on transhydrogenase This enzyme first described by Colowick *et al*[298] catalyses the following reaction Reduced triphosphopyridine nucleotide (TPNH) + diphosphopyridine

nucleotide (DPN) ⇌ Reduced diphosphopyridine nucleotide (DPNH) + triphosphopyridine nucleotide (TPN). Ball and Cooper[88] have shown that this enzyme (in a heart particulate preparation) will catalyse the oxidation of TPNH only if DPN is added to the preparation. L-Thyroxine in concentrations of 4.5×10^{-6} M–4.5×10^{-5} M inhibited the reaction. Although such an inhibition has only been demonstrated *in vitro*, the authors cite other evidence to support the hypothesis that such an inhibition represents the mode of action of thyroxine *in vivo*; they suggest that if transhydrogenase activity is blocked by thyroxine, then the oxidative reactions in body requiring TPN would obtain it from an alternative source—one dependent on a TPNH cytochrome *c* reductase; the finding of Phillips and Langdon[1216] that the level of TPNH cytochrome *c* reductase is increased in the livers of hyperthyroid rats while it is reduced in the livers of hypothyroid animals would fit in with this concept; further, the observation of Drabkin[396] of an increased cytochrome *c* level in hyperthyroid animals would lend support to this view. Ball and Cooper[88] explain the reduced phosphorylating efficiency caused by thyroid hormones by the fact that oxidation of TPNH mediated by enzymes other than transhydrogenase yields little of high energy phosphate compounds[831]. Whether the inhibition of the transhydrogenase reaction, the increase in TPNH cytochrome *c* reductase activity or effects on other enzymes sensitive to the thyroid hormones represents a mode of action of these hormones still remains at best a working hypothesis; it has yet to be shown whether inhibition or activation of these enzymes is not an adaptive mechanism involved to adjust to the effects of a primary action at some other site. However, the theory of inhibition of transhydrogenase is attractive since it implies that the thyroid hormone might control the availability of pyridine nucleotides in the cell. Because of the fundamental importance of these substances for so many biological oxidation reactions, energy utilization and synthesis of body constituents, the effect on the transhydrogenase reaction (or some similar one) might explain why the activity of so many enzyme systems are altered as a result of thyroid hormone administration.

(iv) *Why do the effects of thyroid hormones on enzymes fail to explain their mode of action?*—The idea that hormones control specific key enzyme functions in the cell has been widely accepted as their mode of action. As Hechter[716] has so aptly stated, "Originally advanced as a tentative working hypothesis . . . the hypothesis assumed in many circles the transcendental quality of a natural law. . . . Yet the fundamental inconsistencies are so numerous that some investigators have now come to doubt whether hormone enzyme interaction represents the mechanism whereby their physiological action is effected." Hechter[716] has listed some of the major requisites in order that a theory can explain the mode of action of any hormone; the reader is referred to his essay and to that of Levine[959] for criticisms of the

hormone enzyme theory in general, more detailed criticisms regarding the effects of thyroid hormones on enzymes will be found in the discussions that have followed the papers by Lardy and Maley[905] and Lardy[80, 903]. The major shortcomings of the thyroid hormone enzyme theory are summarized below.

(1) *Lack of specificity of the enzymes affected by thyroid hormones*—One of the important concepts of the role of enzymes in physiological processes is that they are 'pacemakers' or 'bottlenecks'. By this it is meant that a particular biological phenomenon such as oxygen consumption or protein synthesis is the end result of a chain of enzymically catalysed reactions and that the overall rate of the process will be governed by the rate of the slowest individual step in the sequence. It is therefore logical to assume that any hormone, in so far as it is involved at all, controls the rate of a physiological function either by accelerating or inhibiting the rate of the slowest step or 'pacemaker'. So far hormonal control of such a 'pacemaker' has not been demonstrated. As we have already seen, the search for a specific key step involved in the uncoupling of oxidative phosphorylation which might be controlled by the thyroid hormones has proved unsuccessful[905, 1015, 1013]. The concept of an inhibition of one particular phosphorylating step in the sequence leading to partial uncoupling from oxidation (and hence possibly, higher O_2 consumption *in vivo*) has been challenged by Hoch and Lipmann[742]; these authors obtained total inhibition of phosphorylation under certain conditions during the oxidation of glutamate or β-hydroxybutyrate, i.e. a P/O ratio of 0. The enhanced oxidation of succinate by thyroid hormones resulting from the specific inhibition of malic dehydrogenase[1741] does not explain the handling of increased malate thus accumulated[111]. An examination of Tables V and VI which show the very widely differing enzymes that are affected by thyroid hormones makes the search for a specific pacemaker even more difficult and discouraging. This may stimulate efforts to seek a more fundamental process under hormonal control whose alteration would be reflected in effects produced on a large number of enzymes in the cell. A good example of such a process, and one we shall examine later, is the regulation of availability of substrates for enzymes by the modification of permeability of membranes surrounding cells or particulate matter in the cell.

(2) *Absence of a relationship between the physiological activities of thyroxine and related compounds and their actions on enzymes*—In trying to reconcile the action of structural analogues on enzymes and their hormone-like properties in the intact animal, the lack of specificity obtained *in vitro* presents a major stumbling block. There is no correlation between chemical structure and action on enzymes on the one hand and on the whole animal on the other. For example D- and L-thyroxine which have

very different biological activities (see Table II) uncouple oxidative phosphorylation to about the same extent *in vitro*[905, 85, 742] The anti thyroxine drug *n* butyl 4-hydroxy 3 5 diiodobenzoate is as potent an uncoupler as thyroxine itself[411, 742] However Klemperer[857] has obtained a greater degree of uncoupling with L thyroxine than with D thyroxine L 3 5 diiodothyronine or L thyronine none of which possesses much or any biological activity The uncoupling action of thyroxine is not likely to be due simply to the presence of iodine atoms in the molecule as Middlebrook and Szent Gyorgi[1084] have suggested since Klemperer[857] failed to obtain uncoupling with 2×10^{-4} M iodide Middlebrook and Szent Gyorgi[1084] obtained uncoupling by the drastic expedient of substituting all the chloride in their incubation medium with iodide Uncoupling of oxidative phosphorylation can however be caused by a variety of non iodinated substances such as bilirubin[1768] salicylic acid[201] and the free radical tetramethylphenylenediamine[1145] the uncoupling action of these different substances is mediated through different mechanisms and uncoupling of oxidative phosphorylation has been considered by many pharmacologists to represent the mode of action of a large number of drugs[2 0] Further examples of lack of specificity in chemical structure and activity *in vivo* and *in vitro* are provided in the effects of certain biologically inactive substances on the activation of mitochondrial ATPase[905] the physiologically inert DL *ortho* thyroxine and 3 5 diiodothyropropionic acid were as active *in vitro* as L thyroxine and L triiodothyronine With regard to the stimulation of succinoxidase or inhibition of the oxidation of malate Barker[111] has related the activities *in vivo* and *in vitro* of thirty two structural analogues of thyroxine Again no correlation can be found between physiological action and effect on the enzymes For this reason the hypothesis that the thyroid hormones exert specific control on one or more stages of enzymic action is one of doubtful value at the present time

(3) *Does the effect of thyroid hormones on enzymes represent a pharmacological rather than a physiological action?*—In order that we may interpret the effect of a hormone on an enzyme in terms of a physiological process in the intact animal the concentration of hormone used for eliciting response *in vitro* must not be high compared to the physiological level of the hormone in the tissues In the experiments so far discussed the responses have been obtained only in the presence of very high concentrations of thyroxine or related substances or after the animal has been brought to an extreme state of hyperthyroidism For example animals have been made thyrotoxic by feeding them a diet incorporating 1–3% of desiccated thyroid[905, 1015, 101, 979] or by injection of large doses of thyroxine[1041, 411, 742] In some experiments as much as 28 mg of thyroxine has been injected in a rat over a period of 4 days[411, 1569] Such a dose is highly toxic, the animal loses 10% or more of its body weight by the 5th day of

treatment. In order to obtain responses to thyroid hormones in enzyme preparations a concentration of 10^{-4} to 10^{-5}M is generally necessary[1041, 348, 568, 1014, 905, 742, 111, 1741, 86, 1747, 696, 223] Reports that lower concentrations are capable of stimulating succinoxidase[15, 1850, 1851] have not been confirmed. A concentration of 10^{-5} to 10^{-4}M thyroxine is 100–1000 times the concentration of the circulating hormone or probably 10 000–100,000 times that found in tissues such as skeletal muscle It might be argued that such large amounts of thyroxine and triiodothyronine are necessary because they have to be converted to some "active" form of the hormone so far such a conversion either in the whole animal or in isolated preparations has not been demonstrated Also, the converse effect cannot be obtained in enzyme preparations from hypothyroid animals For example the P/O ratio in mitochondria (containing a phosphate acceptor) from hypothyroid animals is not different from that of mitochondria from normal animals Thus it is evident that most of the effects of thyroid hormones on enzymes are a manifestation of their toxic effects and are unlikely to represent events occurring under physiological conditions

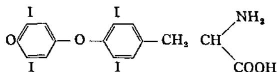
(4) *Difficulty in correlating direct action on enzymes with known physiological action*—Finally the gap between the effects produced on enzymes *in vitro* and the sequence of interactions leading to the physiological properties of thyroid hormones has not been bridged If we consider the relationship between the calorigenic action of thyroxine in the intact animal and its uncoupling effect in mitochondria we find that the rise in oxygen consumption *in vivo* is not accompanied by any significant rise of oxygen consumption *in vitro*, although inhibition of phosphorylation is observed It has been suggested that uncoupling might theoretically lead to increased respiratory activity but this has only been reported once The findings of Wiswell and Braverman[1734] that thyroxine stimulated oxygen consumption in mitochondria or submitochondrial particles are exceptional Further only a few reports have been made of increased respiration of tissues when thyroid hormones are added *in vitro*[348, 257, 1753] Moreover these studies have not been confirmed[103, 110, 1735] Another unanswered question is how far the effects of thyroid hormones on enzyme activities represent a primary site of action or are secondary to some other action If as some recent work already suggests the enzyme effect takes place only after the hormone has acted on some other target, i e by changing membrane permeability or by forming metal complexes then the concept of a direct interaction between thyroid hormone and enzymes is of secondary importance

The above criticisms are not intended to dismiss entirely the current concept of thyroid hormonal regulation of enzyme activity from our thoughts The mode of action of these hormones may indeed be found in the control of certain enzyme systems, however, in order to prove this

point some of the objections and inconsistencies mentioned above will have to be resolved It is likely that our methods of studying the direct interaction between thyroid hormones and enzymes have been inadequate for the purpose our knowledge of the mechanism of enzyme action is in itself incapable of explaining biological phenomena and as Stetten[1541] has recently said It follows almost necessarily, that since we don t know the mechanism of any enzyme catalysed reactions we do not know the mechanism of the endocrine control of such a reaction At present no hypothesis has been put forward to explain the mode of action of the thyroid hormones which is entirely acceptable but interest has recently been turned towards two other factors that may be involved in this mechanism

(b) Interaction with Metal Ions

Metal ions such as Fe^{++}, Mg^{++} Mn^{++} Cu^{++} and Zn^{++} are essential for the activity of many enzymes involved in oxidative and phosphorylating processes and it has been suggested that many of the effects of thyroid hormones on enzymes might be due to their interaction with metal ions Although De Caro in 1933[354] had postulated that the anti oxidative effect of thyroxine on fats was due to a removal of copper the first proper investi gation arose from the observation of Gemmill[564] that thyroxine accelerated the oxidation of ascorbic acid by ascorbic acid oxidase a copper containing protein prepared from squash (*Cucurbita sp*) Gemmill also observed that thyroxine inhibited the non enzymic oxidation by cupric ions in the $CuCl_2$ ascorbic acid system There was a definite relationship between the amount of thyroxine and degree of inhibition of the non enzymic system and it was assumed that thyroxine combined with and removed copper from the oxidative process Gemmill later showed that 3 5 diiodo tyrosine 3 5 diiodothyronine and thyronine which are physiologically inactive had little effect on enzymic or Cu^{++} catalysed oxidations while triiodothyronine was almost as effective as thyroxine[567 569] It was further shown[573] that the most effective molar ratio for inhibition was approximately three molecules of thyroxine to one of cupric chloride Gemmill assumed that complex formation was between copper and a hypothetical free radical of thyroxine of the type



Later from a study of the modification of the ultra violet absorption spectrum of thyroxine in the presence of copper ions Gemmill[570] con cluded that the complex between copper and thyroxine involved the

phenolic hydroxyl group and that maximum change in the absorption spectrum was observed with five parts of thyroxine to four parts of cupric acetate If the complex or chelate involves the hydroxyl group it is difficult to explain why 3 5 diiodotyrosine does not affect ascorbic acid oxidation In fact Frieden[537] who confirmed Gemmill s observation on the effect of thyroxine on ascorbic acid oxidase, failed to demonstrate any specificity of thyroxine for copper complexing and found that 3 5 diiodo thyronine and *O* benzyldiiodotyrosine were equally effective[539] However on the basis of the finding that ascorbic acid oxidase is very readily inhibited by cupric and other metal ions[536] it was suggested that thyroxine activated the enzyme by combining with the excess of copper or other metal impurities Frieden and Naile[539] have also shown that thyroxine prevents and reverses the phenylmercuric chloride inhibition of ascorbic acid oxidase and that it reacts directly with the organic mercurial It follows from these observations that thyroxine may also act on the enzyme by protecting the —SH groups from oxidation This action of thyroxine is also shared by most substances that are powerful chelators of copper such as proteins amino acids and nucleic acid components[539, 538] The work of Davis[349] indicates that the amino acid group of thyroxine may participate in copper complex formation he prepared copper complexes of thyronine and various iodothyronines and iodotyrosines and showed that the empirical formula of all these complexes was Cu(amino acid)$_2$ The formation of copper complexes by thyroxine has little bearing on its mode of action since no copper dependent enzyme in animal tissues has been shown to be affected by thyroxine further whether complex formation would take place at the very low concentrations of copper and thyroxine present in cells is difficult to assess

The interaction between thyroid hormones and magnesium ions appears a more promising field for study because Mg^{++} is an important component of many enzymes involved in oxidation and phosphorylation Partially purified creatinephosphokinase (or ATP-creatine transphorphorylase) an enzyme dependent on Mg^{+} for its activation has been shown to be inhibited by thyroxine[63] This inhibition was also demonstrated on the crystalline enzyme by Kuby *et al* [884, 885] who suggested that the inhibition resulted from the removal of soluble Mg^{++} Lardy[902] has cited the low solubility of Mg salts of thyroxine recorded by Kendall and Osterberg[848] and the low solubility product of 10 ^{17} of the complex with Mg^{+} in support of this view and produced evidence to show that thyroxine also forms complexes with cobalt zinc magnesium manganese and calcium on the basis of a shift in the ultra violet absorption spectrum he concluded that whereas Co^{++} and Zn^{++} combine with the phenolic group of thyroxine Mg^{++}, Mn^{++} and Ca^{++} do not

The formation of an insoluble complex with magnesium does not explain

the observations of Bain[85] on the antagonism between Mg^{++} and thyroxine on the uncoupling of oxidative phosphorylation, it was found that the uncoupling effect of thyroxine *in vitro* could be overcome by increasing the concentration of Mg^{++} in the incubation medium conversely the uncoupling action of thyroxine was accentuated at low concentrations of Mg^{++} These results have been confirmed by other workers[1124, 15 0, 1569, 1734] Mg^{++} was also found to overcome the effect of thyroxine in mitochondria obtained from hyperthyroid rats[1568] This protective effect of Mg^{++} is specific for thyroxine and its derivatives, and antagonism is absent when uncoupling of oxidative phosphorylation is brought about by other agents such as dinitrophenol and Ca^{++} ions Thus Mg^{++} failed to overcome the uncoupling of oxidative phosphorylation when both thyroxine and Ca^{++} ions were present[1734] The antagonism is also observed for triiodothyronine and triiodothyroacetic acid[1124] which do not form insoluble complexes with magnesium The form in which these complexes exist has not so far been unequivocally demonstrated however it appears that the thyroid hormones and some of their analogues are prevented from acting on the mitochondria whether the magnesium complexes are soluble or insoluble Manganese and cobalt can replace Mg^{++} to some extent[1123, 1568, 1734] and the protective action on uncoupling is also shared by *glutathione and ethylenediaminetetraacetate* (EDTA)[1194, 1569] The mechanism of the antagonistic effects of the divalent metal ions glutathione and EDTA on uncoupling by thyroxine is unknown but two suggestions have recently been put forward Park *et al*[1194] have reconsidered the formation of a free radical of thyroxine similar to that proposed by Gemmill[567] and have based their views on the interesting observation that agents which protect mitochondria against uncoupling by thyroxine also protect cells against damage by X irradiation a damage widely believed to be caused by the formation of free radicals Tapley[1567, 1569] believes that magnesium antagonizes thyroxine by preserving the mitochondrial structure (see below)

There is some indirect evidence that magnesium metabolism is altered in humans with thyroid disorders[1499, 1498, 378] but this has not always been substantiated[307, 170, 1464] Tapley[1566] has however shown that the administration of triiodothyronine to patients with myxoedema causes a marked rise in the urinary excretion of magnesium As regards the Mg^{++} content of mitochondria themselves very little difference has been found in samples prepared from hyperthyroid hypothyroid and normal rats although the mitochondria from the hyperthyroid animals stimulated ATPase a Mg^{++} activated enzyme[1012] On the other hand the work of Vitale and his colleagues indicates a direct relationship between thyroid hormone administration and Mg^{++} metabolism they first reported that increased Mg^{++} in the diet prevented uncoupling of oxidative phosphorylation

in hyperthyroid rat heart mitochondria (which they found were more sensitive to Mg^{++} and thyroxine than liver or kidney mitochondria) and they inferred that thyroxine raised Mg^{++} requirement, this view was substantiated by the low serum Mg^{++} and typical magnesium deficiency symptoms in hyperthyroid animals[1688] In a later paper, Vitale *et al*[1697] showed that in young rats magnesium deficiency alone produced uncoupling of oxidative phosphorylation within a few days they further claimed that an acute effect of thyroxine in lowering the P/O ratio (within 10 min after administration) could be largely prevented by a single injection of magnesium 10 min before the administration of the thyroxine Such a rapid action of thyroxine on mitochondria has not previously been reported

Little is known about the interaction of thyroid hormones with other metal ions An interaction between Zn^{++} and thyroxine has been suggested as one of the explanations for the inhibition by thyroxine and its analogues of glutamic yeast alcohol lactic, malic, triosephosphate and glucose 6 phosphate dehydrogenases, enzymes which show varying degrees of zinc dependence[1747] However, no direct evidence of a Zn^{++} thyroid hormone chelation has been demonstrated and triiodothyroacetic acid failed to inhibit carboxypeptidase which is a zinc containing enzyme Further, acetyl phosphatase which is powerfully inhibited by thyroxine, is not dependent on any of the following metal ions for its activation Mg^{++}, Mn^{++}, Cu^{++} Co^{++}, Fe^{++}, Fe^{+++} Zn^{++} or Ca^{++}[686] It may be concluded that all the effects of thyroid hormones on enzymes are not manifestations of an interaction between the hormones and metal ions

(c) *Effect on Membrane Permeability*

The increasing difficulty of relating the action of any hormone *in vivo* to its action on subcellular preparations and isolated enzymes has made workers seek for other explanations in recent years for the mechanism of hormone action in particular a possible role of the membranes that surround cells nuclei and mitochondria has been considered Enzymic processes in isolated preparations depend mainly on enzyme and substrate concentrations the hydrogen ion concentration energy donors and acceptors and inorganic ions in the cell these processes are further controlled by structural and surface phenomena of the membrane These have been called cytoarchitectural cytostructural or geometrical aspects of biochemistry This concept of the mode of action of hormones owes its origin to studies on insulin Until a few years ago mainly because of the work of Cori and his associates it was generally accepted that insulin exerts a direct action on glucokinase which controls the conversion of glucose to glucose 6 phosphate and hence the formation of glycogen and the oxidation of glucose to CO via the tricarboxylic acid cycle However, the discovery by Gemmill[564 565] that insulin increases the uptake of

glucose by diaphragm muscle *in vitro* led to numerous experiments by Levine and others on the permeability of membranes to different sugars and considerable evidence has accumulated in favour of the view that insulin acts by accelerating the transfer of glucose across the cell membrane This transition from the concept of a direct hormone enzyme interaction to a hormone membrane interaction has been illustrated in numerous reviews and papers[204, 476, 1515, 1518, 860, 714, 1701, 1384, 288, 958, 1597, 318]

No such change of thinking has taken place as regards the mode of action of thyroid hormones and little has been done to determine the effect of these hormones on the properties of cell membranes moreover this work has been directed towards the effect of thyroxine on the transfer or utilization of glucose on the same lines as the insulin work this has not resulted in the demonstration of any significant control of tissue permeability by thyroid hormones although they do accelerate the overall utilization of glucose[757, 340, 349, 997] The only exception is the recent brief report by Comsa[301] that the rate of glucose uptake by rat diaphragm in the presence of 10^{-7} to 10^{-6} M thyroxine was 179–310% of the control value If this is confirmed an extension of this work on other substrates more directly involved in oxidative processes may prove profitable

This lack of evidence on a direct action of thyroid hormones on the permeability of cell membranes has however been offset by recent work from Lehninger s laboratories which has already received much support In 1955 Tapley *et al*[1570] suggested that the uncoupling of oxidative phosphorylation induced by thyroxine in mitochondria is not a result of a direct interaction of the hormone with enzymes of oxidative phosphorylation but that it occurs indirectly as a result of the effect of thyroxine on the structure of mitochondria. This suggestion was followed by two detailed reports which explain to a large extent all the effects of thyroxine and related substances on oxidative phosphorylation which have previously been described[1587, 1588] In the first report the effect of various agents that caused swelling of mitochondria was studied no oxidation or phosphorylation occurred in these experiments and the swelling represented only a passive diffusion of water into the particles Some uncoupling agents such as thyroxine and Ca^{++} caused a rapid swelling of mitochondria under these conditions while others such as 2 4-dinitrophenol dicoumarol and pentachlorophenol had no effect or even prevented mitochondria from swelling Agents which antagonized the thyroxine induced uncoupling of oxidative phosphorylation like Mg^{++} Mn^{++} and EDTA also antagonized the mitochondrial swelling caused by thyroxine Mg^{++} overcame the effect of antagonizing the effect of thyroxine on mitochondrial structure rather than by chelation as previously suggested Concentrations of thyroxine as low as 10^{-8} M caused swelling such a concentration would not lower the P/O ratios Tapley also showed that liver mitochondria prepared

from thyroxine treated rats swelled to a greater extent than those from normal rats, while the swelling of mitochondria from hypothyroid animals was much diminished In the second report Tapley and Cooper[1568] have provided further evidence to support the belief that the effects of thyroid hormones on oxidative phosphorylation might be the result of changes in permeability of mitochondria they prepared an active enzyme complex capable of catalysing oxidative phosphorylation from mitochondria after the membranes had been disrupted by treatment with digitonin, whereas thyroxine and triiodothyronine lowered the P/O ratio in intact mitochondria in hypotonic sucrose without any preincubation the two compounds failed to uncouple oxidative phosphorylation in the digitonin extracts of mitochondria in any concentration In contrast dinitrophenol uncoupled phosphorylation just as effectively in intact mitochondria as in the extracts once again demonstrating that DNP and thyroid hormones act by totally different mechanisms However Bronk[223] and Park *et al* [1193] have recently demonstrated an uncoupling of oxidative phosphorylation by thyroxine and triiodothyronine (but not by 3 5 diiodothyronine) in submitochondrial particles prepared by sonic treatment[224] These differences may be due to variations in the degree of disintegration produced by digitonin and sonic treatment or to the presence of digitonin itself

The swelling or modification in structure of mitochondria caused by thyroid hormones had also been previously observed by others[13, 742, 858] but the significance of these changes was not discussed Aebi and Abelin[13] thought that the increased rate of loss of potassium ions from liver slices obtained from thyrotoxic animals was secondary to an impaired phosphorylation rather than to a primary action of thyroid hormones on active or passive transport mechanisms in mitochondria The work of Tapley [1567, 1569] has now been confirmed and extended[377, 265, 1738, 434, 306, 934, 935, 433] Maley[1012] has reported that mitochondria isolated from hyperthyroid rats were more fragile than those from normal animals Effects on the mitochondrial membrane are also manifest in the study of ATPase activity of mitochondria Klemperer[880] observed that ageing caused swelling of mitochondria and thyroxine had lost its stimulatory effect on latent ATPase DNP on the other hand activated ATPase in both fresh and aged mitochondria Cooper and Lehninger[305] have shown more directly that thyroxine and triiodothyronine unlike DNP or pentachlorophenol, do not stimulate ATPase activity of digitonin extracts of mitochondria Among some of the interesting findings that have emerged from these studies is that of Tapley and Cooper[1569] who found a very marked parallel between mitochondria from different tissues that were susceptible to swelling and to an increased respiration caused by thyroid hormones They found that thyroxine *in vitro* produces swelling in mitochondria from liver and kidney but not in those from spleen brain and testis in hyperthyroid

rats liver and kidney show an increase in O consumption while spleen, brain and testis do not The nutritional status also appears to control mitochondrial swelling, the response of liver mitochondria from normal animals is much greater than from fasted animals Emmelot and Bos[434] have reported that mitochondria isolated from primary azo dye induced liver tumours of mice and rats have almost completely lost the ability to swell in the presence of thyroxine They conclude that thyroxine has to interact with some component of the integrated biochemical structure of liver mitochondria

Very little is known about the mechanism or factors controlling the effect of thyroid hormones on the mitochondrial membrane structure Beyer *et al*[165] suggest that thyroxine makes the mitochondria more susceptible *in vivo* to factors which may lower their phosphorylative efficiency, according to Emmelot[433] the primary effect of thyroxine consists in sensitizing the mitochondria to Ca^{++} or some other metal ion The oxidation reduction state of mitochondria has been shown to be the primary determinant of the passive swelling process[934, 935] These authors found that the swelling caused by thyroxine and triiodothyronine is completely inhibited when the respiratory carriers are in the reduced state (i e under anaerobic conditions or in the presence of KCN) Further with refinements of techniques Lehninger and Ray have detected swelling caused by thyroxine at a concentration of 10^{-8} M which approaches the physiological range

The work of Tapley Cooper and Lehninger has thus presented a new sphere of activity in which the control of respiration by thyroid hormones may be manifested This work has not yet explained the exact mechanism of action of thyroid hormones, but it is reasonable to believe that their effects on enzymes are indirectly brought about by an action on the structure of the mitochondrial membrane thus controlling the entry and exit of substances involved in respiratory activity and energy transfer At the same time many new questions have to be answered What is the nature of the sites on the membrane that respond to thyroid hormones? How do they function? Is there a binding of thyroid hormones on the membrane? This question might be studied in the same profitable manner as that of insulin binding on the cell surface[1515] Is the action of thyroid hormones on membranes definitely a primary action? Finally are these only pharmacological effects or can they be shown to occur in the intact animal and can they explain all the physiological actions of thyroid hormones?

CHAPTER 7

EXTRATHYROIDAL DISTRIBUTION AND METABOLISM OF IODINE

EARLY studies on the distribution and metabolism of iodine have been reviewed by Loeb[981] Marine[1024] Elmer[427], Salter[1404 1405] and Leblond[919] Before the publication by Chaney[275] of a micromethod for determination of iodine suitable for the estimation of fractions of a microgram of iodine doses of iodine far in excess of a physiological range had to be administered to animals in order to study its extrathyroidal metabolism Fortunately within a few years of Chaney s publication, the introduction of radioactive iodine as a tracer ' has also contributed to the study of the problem under more physiological conditions

The clinical interest in the physiology and diagnosis of various thyroid disorders has greatly stimulated the study of dynamic aspects of the extra thyroidal fate of hormonal as well as inorganic iodine The use of radio active iodine especially ^{131}I, in the diagnosis and treatment of thyroid diseases is now a standard procedure in many hospitals and research institutions and date sfrom the first experiments of Hamilton and Soley[671] in 1939 While this clinical work cannot be fully considered here (see [731 839 1143 1305 158 465 1164 826] *an effort will be made to assess the* relation ship between the pathways of metabolism of the thyroid hormones and their physiological actions

(I) EXTRATHYROIDAL DISTRIBUTION AND METABOLISM OF IODIDE

(a) *Distribution and Concentration*

In general the distribution of iodide in body tissues and fluids resembles that of chloride and bromide and also that of thiocyanate[1691 1758] Because of a very rapid absorption through the duodenum and intestine[832 296 1201] the blood level of orally administered iodide reaches its peak within 1 hr of ingestion It then falls exponentially the rate of fall being mainly dependent on the activity of the thyroid gland This is the basis of an indirect clinical test in the diagnosis of thyroid diseases in which the rate of fall of blood ^{131}I is determined[839] (Fig 16)

The thyroid gland is the principal iodine concentrating organ in the body However circulating iodide is also concentrated by salivary gastric and mammary tissues because of this extrathyroidal concentration of iodide, Myant and his colleagues have observed an apparent expansion of iodide space in man (the iodide space is defined as the amount of ^{131}I

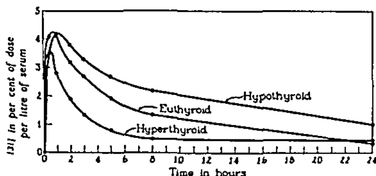


Fig 16

Average curves of serum ^{131}I content in groups of human subjects with varying thyroid functions during the first 24 hr administration of ^{131}I The availability and distribution of iodide in extrathyroidal sites depend upon the thyroid function of the individual (From Keating and Albert[888])

remaining in the body as per cent of dose per litre of plasma) This expansion was from 18 l in the 1st hr after ^{131}I administration to 25 l in the next 5 hr[1141] The twenty- to forty-fold iodide concentration in saliva, gastric juice and milk compared with the plasma level supports the concept of an expansion of iodide space This is particularly noticeable when the thyroid gland is blocked with thiouracil[16 0] In recent years the mechanism of iodide concentration at these extrathyroidal sites has been shown to be identical with that in the thyroid gland, both in man[1141 756 532 576 553 1533 1603] and in lower animals (rat mouse guinea pig hamster rabbit dog and goat) The rat in contrast to the mouse shows an absence of salivary concentration of iodide[814 1754 493 533 665 230 773 721 246] Such a conclusion was reached from experiments based on effects produced by variation of the iodide gradient and the administration of substances that block thyroid function namely, thiocyanate and perchlorate ions The phylogenetic origin of the thyroid gland from the anterior portion of the primitive gut makes this similarity in both qualitative and quantitative aspects of iodide trapping by thyroidal and extrathyroidal tissues particularly interesting As is well known the activity of the thyroid gland is controlled by the anterior pituitary hormone thyrotrophin it has yet to be shown whether the extrathyroidal iodide concentrating mechanisms are also controlled by thyrotrophic activity of the pituitary gland

Iodide penetrates into most other tissues and body fluids but its physiological significance with a few exceptions is doubtful[814 756 1 03 1655 38 665 1636] A constant ratio of 0 565–0 670 in the distribution of iodide between erythrocytes and blood plasma of many vertebrates has been repeatedly observed[1262 185 1712 319] and has been used in some cases for the indirect measurement of thyroid function Fontaine and Leloup[511]

have observed curious differences in this erythrocyte–plasma ratio in some cold blooded vertebrates The ratio in the salmon is as low as 0 09, for the carp and trout it is 0 20–0 35 it rises to 0 52 in the eel and other Protoptera The existence of a mechanism for making iodide available to embryonic or foetal thyroid tissues has been known for some time Thus in mammals a favourable placental permeability to iodide leads to a higher concentration of ^{131}I in foetal than in maternal blood after the administration of radioactive iodine to the mother[656, 1147, 544] In birds, concentration of iodide by the ovaries results in relatively iodine rich vitellin oocytes and egg yolk[372, 1368]

(b) *Excretion of Iodide*

Except for a small amount of inorganic iodine lost in the sweat[474] the major route of excretion is through the kidney Skanse[1477] illustrated this by recovering 98% of administered ^{131}I in the urine of a thyroidectomized subject In the first 6 hr after ingestion the thyroid gland and kidney are actively competing for iodide ions Thus the rate of renal clearance of iodide which is about 0 1 ml/min in the normal rat is nearly doubled if the thyroid gland is blocked by propylthiouracil[2, 7] This competition for iodide ions between the thyroid gland and kidney forms the basis of a complementary clinical test in the diagnosis of thyroid diseases this consists in measuring urinary ^{131}I as a function of time after ^{131}I administration[939, 1141, 1305, 1064, 1171]

(c) *Extrathyroidal Conversion of Iodide to Organically Bound Iodine*

Since some extrathyroidal tissues possess the same iodide trapping mechanism as the thyroid gland the question is whether these can also incorporate iodine into organic linkage The formation of iodoprotein by mammary and gastric tissues has been described only recently The mechanism seems similar to that in the thyroid since thiouracil inhibits the formation of protein bound iodine in slices and particulate fractions of mammary tissue[533, 287, 1588] The iodoprotein found in milk yields after hydrolysis monoiodotyrosine together with traces of diiodotyrosine thyroxine and triiodothyronine on the other hand have never been detected[1568, 1243, 234] The extent of organic binding of iodine is not great and does not exceed 5–10% of total iodine It has been reported however, that in the milk of Japanese women the protein bound iodine may be as high as 2 36 μg/100 ml[714] The iodination of proteins in gastric tissue has not been adequately described except in the case of the peptic gland tissue of the rat stomach[522] and the Heidenhain pouch of the dog[713] Only inorganic iodine has been detected in the saliva or salivary gland although Fawcett and Kirkwood[468] have claimed a special function for the salivary gland in the metabolism of iodine However there has been no confirmation of the

presence of a "tyrosine iodinase" nor of any deiodinating mechanism in the salivary gland[1398, 1150]. The physiological significance of organic binding of iodine in stomach and mammary gland remains unknown. It is however, interesting to note that iodination of protein in these tissues stops at an elementary biosynthetic stage, i.e. the formation of monoiodotyrosine; diiodotyrosine is absent or present only in traces. Such iodoproteins are also characteristic of embryonic, neoplastic or pathological thyroid tissues (see Chapter 3). The principal features of extrathyroidal iodine metabolism are summarized in Table VII.

TABLE VII

Extrathyroidal iodide (I^-) trapping sites in mammals

Site	Species	I^- concentrated or secreted	Tissue/serum I ratio	Whether iodine organified	References
Salivary gland	Man	Saliva mixed	30	—	1141
		Parotid juice	>40	—	6
	Mouse	Salivary gland *in vitro*	5–10	No	4 2
	Dog	Submaxillary gland	<1.6	—	46
	Man	Saliva	17	—	5
				No	1398
Stomach	Man	Gastric juice	30–40	—	1141, 766
	Hamster	Gastric juice	26	No	9 4
	Mouse	Gastric juice	6	No	864
	Dog	Gastric juice	10–50	Yes	772, 773
Mammary gland	Man	Milk	15–30	—	75
	Man	Milk	—	Yes	716
	Rabbit	Milk	7–38	Yes	231, 234
	Rat	Milk	—	Yes	
	Rat	Mammary gland slices	1–2	Yes	
Placenta	Rabbit	Foetal blood	2–3	No	846

(d) *Is there an Extrathyroidal Synthesis of Thyroid Hormones?*

In 1941 Chapman[277] suggested that thyroid hormone might be elaborated in tissues other than the thyroid gland. His suggestions were based on the observation that thyroidectomized rats maintained on a high iodine diet exhibited a slightly higher oxygen consumption than animals on a low iodine diet. Later, Morton and his colleagues reported the presence of

thyroxine, characterized by a non specific method based on solubility in butanol, in various tissues of surgically thyroidectomized rats[1116] Since then, neither of these reports has been confirmed and no new evidence has been produced to show that thyroid hormones can be synthesized in any but thyroidal tissues The claim of Cetini and Rossetti[267] that inorganic iodine is converted to thyroxine by simple incubation with blood plasma is also unconfirmed although similar incubations have been performed by other workers nor has a similar reaction been demonstrated in the intact animal Although inorganic iodine can be incorporated by extrathyroidal tissues into iodoproteins containing mono- and di iodotyrosines, the elaboration of the thyroid hormone is only effected by the thyroid gland

(II) Distribution and Metabolism of Thyroid Hormones

In most of the early work on the metabolic fate of thyroid preparations or thyroxine, their distribution was studied by chemical estimation of iodine or by biological assay However, lack of accuracy and specificity in the methods made them far from satisfactory[4, 7, 819] Such studies have been considerably facilitated by the use of ^{131}I labelled thyroxine, first prepared by Joliot *et al* [819] and later by the application of chromatography to the separation of iodinated amino acids[483] The discovery of 3 5 3 triiodothyronine and its high biological potency have stimulated interest in metabolic studies of this hormone in the body and have led to a search for other metabolites capable of provoking cellular effects similar to those of thyroxine In this section, qualitative and quantitative aspects of the distribution and metabolic fate of thyroxine and 3 5 3 triiodothyronine will be considered The metabolism of 3 3 -diiodothyronine, 3 3 5 triiodothyronine 3 5 3 5 tetraiodothyroacetic and 3 5 3 triodothyro acetic acids has also been included for purposes of comparison, although their physiological significance is not yet clear

(a) *Some Observations on the Use of ^{131}I labelled Thyroid Hormones*

The advantages of ^{131}I labelled thyroid hormones for the study of their distribution and metabolism are many and obvious, perhaps the most important is the possibility with compounds of high specific activity, of working in a physiological range At the same time it is necessary to consider some of the pitfalls that may be encountered with ^{131}I labelling before a true evaluation of the results can be made

The position of the labelled iodine in the molecule is important in this connection At the present time, most workers use labelled thyroxine and triiodothyronine which are prepared by introduction of ^{131}I into 3 5-diiodothyronine or 3 5 3 triiodothyronine[337], and which therefore are only labelled in the 3 and/or 5 positions[819, 635] Similarly when labelled hormones are obtained by an exchange reaction[585, 1583] the

labelling will also occur only in the 3′ and 5′ positions Some investigators, however, prefer biologically labelled compounds, synthesized after ^{131}I administration in the thyroid gland *in vivo*, since all iodine atoms in these will be labelled[636, 1143], it has been shown that in man biologically labelled thyroxine disappeared from plasma more slowly than did thyroxine which was labelled only in the 3 and 5 positions It appears therefore that the rate of metabolism of the iodine atoms in the two rings is different This difference was even more evident when the rate of urinary excretion of ^{131}I was investigated in rats which had been injected with the two types of labelled thyroxine[1380] Unless a knowledge of the specific activity of the compounds is required the use of biologically labelled material is obviously to be preferred

Once deiodination of ^{131}I labelled thyroxine has occurred it is no longer possible to follow its metabolic pathways For this reason thyroxine labelled with ^{14}C on the carboxyl group has also been investigated[980, 883, 884], unfortunately the feeble specific activity of this ^{14}C labelled thyroxine compelled these workers to use extremely large doses of thyroxine thereby making any interpretation of their results in physiological terms almost impossible

Although material of high specific activity is required for the maintenance of physiological levels of administered hormones a limit is reached when the labelled compound breaks down as a result of self irradiation The instability of solutions of radioactive thyroxine triiodothyronine and iodinated proteins has already been reported[1759, 1760, 970, 1536] Recently Tata[1576] has suggested that the principal degradation products of thyroxine and triiodothyronine from self irradiation may be their lactic acid analogues On chromatographic analysis these compounds closely resemble their thyropropionic thyroacetic and thyroformic analogues the recent claim by several groups of workers that tetraiodothyroacetic acid and triiodothyroacetic acid are formed from the parent amino acids *in vivo* and *in vitro* is therefore not easy to confirm The danger of attributing any biological significance to such a radiochemical reaction needs no further comment The prevention of radiochemical decomposition can nevertheless be facilitated by the storage of material of high specific activity in the frozen state or in the presence of protective substances such as cysteine glycine and serum albumin

(b) *The Overall Rate of Metabolism of Thyroid Hormones*

The experiments of Joliot *et al*[8, 9] qualitatively confirmed the earlier observations that the blood iodine level returned to normal within 24 hr after administration of large doses of thyroid gland preparations[6, 1680] or of thyroxine[59, 60, 190, 1127] The first detailed studies are due however to Gross and Leblond[635] who found that 2 hr after intravenous administration

of rather large doses (up to 2 mg) of labelled DL thyroxine to the rat not more than 2% of the radioactivity was detectable in the plasma. The rate of disappearance was later found to be less rapid when thyroxine of high specific activity was used[839, 16, 20, 636, 285, 1142, 814, 235, 1633]. It appears therefore that large doses of administered thyroxine are more rapidly excreted than small ones. Further, Rall *et al* [1263] have shown that L and DL thyroxine are excreted at different rates, so that no comparisons can be made in experiments in which optically active and racemic thyroxines are used. Other complicating factors are important species differences in the metabolism of thyroid hormones particularly in the enterohepatic circulation and in the kidney these will be considered fully in a later section of this chapter. Lastly, differences in the excretion rates of endogenously and exogenously labelled thyroxine in man and in the rat have been found [114, 814, 679], *although Gross and Leblond*[636] *and Brown Grant* and Gibson[235] failed to observe any marked differences in the rat and rabbit. These discrepancies could be due to contamination of the thyroxine with small amounts of triiodothyronine, since the metabolic turnover of the latter is much faster than that of thyroxine. In any study of the dynamics of thyroxine metabolism it would therefore be preferable to use only labelled thyroxine prepared from non radioactive triiodothyronine.

Quantitative comparisons of the metabolism of thyroxine and triiodothyronine will now be considered. In man the slowest component of the rate of loss of ^{131}I (the slow component reflects actual degradation whereas the initial more rapid rate reflects distribution into tissues) from blood gives a half life time of about 6–11 days for radioactive thyroxine. The half life for triiodothyronine under identical conditions is only 2–3 days [145, 1275, 1263, 1539, 156, 789]. A more rapid disappearance of triiodothyronine has also been found in the rat and mouse[840, 1008, 632, 468].

If we now consider the relative half lives (50% retention time) of thyroxine and triiodothyronine in different species we find marked variations in the rat the values for $T_{\frac{1}{2}}$ are found to be 16 6–19 0 hr for thyroxine and 9–10 hr for triiodothyronine[1008, 468]. The guinea pig and rabbit occupy an intermediate position between the rat and man with respect to thyroid hormone metabolism. $T_{\frac{1}{2}}$ of thyroxine varies between 3–8 days for the rabbit and about 3–4 days for the guinea pig[236, 1633].

To what factors can species differences in the rate of metabolism of thyroid hormones be attributed? The answer must depend on the physiological function of thyroid hormones. As was seen in Chapter 5 their most fundamental physiological role in adult animals is the maintenance of metabolic rate or heat production. Since smaller animals have to maintain a relatively higher metabolic rate than larger animals more thyroid hormones would be needed by the systems involved in heat production. In fact such a [illegible] does exist between the rate of heat production per total body

weight or the pulse rate and the rate of degradation of thyroid hormones in the different species, this was shown in Fig 9

Factors which affect the metabolic turnover of thyroid hormones—The rate of metabolism of the thyroid hormones depends upon the general metabolic state of the animal In the hyperthyroid state the hormones are metabolized more rapidly than in the normal animal in the hypothyroid state, they are metabolized more slowly Hence the biological half life of these hormones can have no real meaning unless reference is made to the animal s metabolic level

Numerous examples of factors which modify the thyroid hormone s half life in man are now available in the extensive literature on protein bound iodine (PBI) determinations in blood for clinical purposes The PBI value represents the difference between the amount of thyroid hormone secreted by the gland and the amount peripherally metabolized or excreted during the same period of time These studies have recently been extended to the investigation of the turnover of ^{131}I labelled thyroid hormones It has been shown that the amount of hormonal iodine utilized daily in the human can be expressed by the equation $D = 2\ 9\ (PBI)^2$ where $D = \mu g$ hormonal iodine degraded per day and $PBI = \mu g$ protein bound iodine per 100 ml plasma.

Among the principal factors that affect the utilization of thyroid hormone are the state of the thyroid gland itself pregnancy and various stresses such as violent muscular exercise and cold In thyrotoxic patients the turnover of thyroxine is greatly increased, but is diminished in patients with untreated myxoedema[789, 1538] A reduction in the rate of utilization of thyroxine and triiodothyronine has been observed in thyroidectomized and hypophysectomized rats and also in rats treated with propylthiouracil [1662, 1663] In laboratory animals the level of dietary iodine is also an important factor in the modification of hormonal iodine metabolism thus Triantaphyllidis[1633] observed a longer biological half life for thyroxine in the rabbit than did Brown Grant and Gibson[236] she attributed this difference to the fact that her animals were maintained on a low iodine diet whereas those of Brown Grant and Gibson were not In the human pregnancy is the only physiological condition which is regularly accompanied by an increase in the concentration of circulating thyroid hormone [70] this is not however found in all animals[1, 15] The suggestion that increased PBI in pregnancy results from an increase in the concentration of thyroxine binding protein[347] has now been proved to be correct[394, 1320, 1313] A similar condition could be simulated by the administration of diethylstilboestrol to normal men and women[437] however Feldman[468] observed that in rats oestrogen administration did not affect the disappearance rate of either endogenous or exogenous thyroxine and triiodothyroxine During exposure of animals to cold it is likely that tissues

require an increased supply of thyroid hormone for maintenance of a normal metabolic rate. Thus, an enhanced rate of utilization of thyroid hormone is found together with an enhanced hormone secretion by the gland[447, 796, 535]. In rats violent muscular exercise caused an increased rate of disappearance of thyroxine; this was also seen after stress caused by adrenaline[449, 93, 447]. Dinitrophenol has also been shown to increase utilization of circulating thyroid hormone, possibly through stress[94, 93]. Escobar del Rey and Morreale de Escobar[450] find that after injection of labelled thyroxine in the rat, the radioactivity is much higher in the gastrointestinal tract of dinitrophenol treated animals than in controls.

(c) *Distribution of Thyroid Hormones in Tissues*

When labelled thyroid hormones are administered by intravenous route to animals, there is an initial rapid loss of radioactivity from the blood followed by a slower loss. The first part of the disappearance curve represents a selective distribution of the hormones in the tissues. In man, Myant and Pochin[1142] found that the thyroxine space* rose rapidly to 23% of the body weight within 6 hr of administration of 3′ 5′ labelled thyroxine and then increased more slowly for the next 18 hr. In a similar study[1263] the volume of distribution of thyroxine during the slower phase on extrapolation to zero time gave a value of about 20% of the body weight. On the other hand, biosynthetically labelled thyroxine had an apparent distribution of 12–15%, when measured over a period of 2–3 days[1539, 156]. The volume of distribution of D thyroxine is twice that of L thyroxine while that of L triiodothyronine is six times as great, exceeding the body weight; this can only be explained by assuming a recycling of the iodide which is more rapidly removed from triiodothyronine and the unnatural isomer of thyroxine. However, actual measurements of the distribution of labelled hormones have only been made in smaller animals; here species differences are less marked in the enterohepatic circulation and in excretion.

Localization of thyroxine in tissues was first studied qualitatively by Kendall[845]; recent work has yielded quantitative information on this subject[635, 636, 637, 638, 17, 1661, 632]. The similarities and differences in the distribution of thyroxine and 3 5 3′ triiodothyronine in the more important tissues are illustrated in Fig 17. The curves represent average values derived from data in the literature cited above.

The differences in rates of loss of thyroxine and triiodothyronine from blood and their appearance in different tissues can easily be seen since the abscissae in Fig 17 are represented in hours for thyroxine and in minutes for triiodothyronine. On the other hand, similarities in the selective distribution of both hormones at different levels are also evident. In general

* Thyroxine space = (percent of dose left in body tissues)/(percent of dose in plasma per litre)

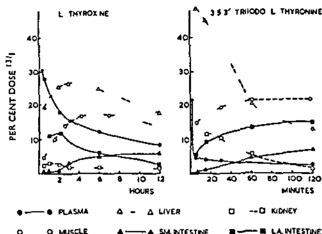


Fig 17

Average curves for the distribution of total radioactivity in plasma and certain organs of the rat after the administration of ^{131}I labelled L thyroxine and 3 5 3 triiodo L thyronine The more rapid disappearance of triiodothyronine from blood and its more rapid entry into all tissues relative to that of thyroxine is seen in the different time scales used in the figure for the two compounds

the tissues to which the thyroid hormones have access can be divided into two categories those in which radioactivity is rapidly concentrated followed by a sharp decrease (liver kidney and stomach) and those in which the radioactivity increases slowly (intestines muscle and skin)

The importance of the liver in the first phase of the metabolism of thyroxine and triiodothyronine is also apparent its role in the transport of iodinated products into the intestine will be examined in detail later on The concentration of thyroid hormone radioactivity in all organs other than liver and also bile is lower than in the plasma However on a total weight basis significant amounts of thyroxine enter into skeletal muscle but the kidney only concentrates small amounts of hormonal iodine it does however dispose of iodide liberated peripherally from the hormones during the early phase of distribution in tissues and of iodide initially present as a contaminant of the labelled preparation

The hypophysis and central nervous system—Many years ago Schittenhelm and Eisler[14 1] showed that there was a relationship in man and other animals between blood thyroxine levels and the iodine content of certain regions of the central nervous system such as the mid brain and tuber cinereum Other investigators[1547] also observed a high concentration of hormonal ^{131}I in the rabbit hypophysis after injection of thyroxine this hypophyseal concentration has since been confirmed with ^{131}I labelled thyroxine[8 0 317 318] The pituitary glands of man and dog also concentrate thyroxine but those of the mouse and guinea pig do not[635 636]

[1588, 442] More recently however a concentration of endogenous (labelled) hormone in the pituitary glands of rats and guinea pigs has been described [1548, 1549] The selective localization of radioactive thyroxine in the neuro hypophysis of rabbits and the feeble concentration in the anterior pituitary is well illustrated by the autoradiograph shown in Fig 18[808]

The concentration of triiodothyronine by the posterior pituitary is much more rapid and intense than that of thyroxine Moreover the difference is more marked in this organ than in any other In the first 2 hr after injection of thyroxine the concentration of radioactivity in the pituitary glands of rabbits is about three times the plasma concentration, with triiodothyronine it rises to from thirty five to forty five times the plasma level [1588, 432, 516, 321, 515] The concentrations of other iodinated compounds i e 3 3 diiodothyronine 3 3′ 5 triiodothyronine and 3 5 3 triiodo thyroacetic acid in the rabbit neuro hypophysis have also been studied[3, 1] An interesting dissociation between the localization of L- and D thyroxine in the posterior pituitary and the biological activity of these isomers is seen in a recent study both optical isomers were concentrated to the same extent, the latter isomer has however no biological significance[322] Localization of endogenous thyroid hormone in the posterior pituitary and surrounding regions in the rat and guinea pig has been demonstrated by a combination of specific radioactivity measurements and autoradiography during the iodide phase' (2 hr) and hormone phase' (48–72 hr) after a tracer dose of ^{131}I [1548, 1549], chromatographic analysis showed that most of the thyroxine and triiodothyronine largely remained unchanged[809, 1588, 515] in the posterior pituitary although some deiodination may occur[1484] Jentzer has also shown[809] that the presence of the thyroid gland is not necessary for the localization of thyroxine in the neurohypophysis

The concentration of thyroid hormones in the posterior pituitary is a phenomenon which may have no physiological significance, since it is the anterior pituitary which controls the activity of the thyroid gland It is, however, possible that the neurohypophyseal localization of thyroid hormones regulates the production of thyrotrophic hormone in the anterior pituitary Heinbecker[719] has in fact shown that the number of basophil cells of the anterior pituitary is controlled by the neural hypophysis It is of interest to note that another endocrine secretion cortisone is also selectively localized in the posterior pituitary[323] and that cortisone has been known to suppress thyroid activity Hence the specific localization of thyroid hormones in the posterior pituitary could represent a part of the pattern of auto regulation of levels of endocrine secretion of glands controlled by the pituitary

The concentration of hormonal iodine in other regions of the central nervous system, with the exception of the hypothalamus is about twenty



Fig 18

Selective localization of ¹ I labelled L thyroxine in the po tenor hypophysis of the rabbit The photom crograph of the section (below) and the corresponding autoradiograph (above) clearly demonstrate the concentration of radioact ity in the neural lobe (From Jensen and Clark)

to sixty times less than in the posterior pituitary However definite localization of labelled thyroid hormone has been observed in the cerebral cortex and to some extent in the cerebellum[1548 1549 35 515] It is worth commenting on the fact that the regions of localization of thyroid hormones in brain tissue correspond with the distribution of deiodinating activity described by Tata[1573 1574] The low concentrations of hormonal iodine in brain tissue compared with blood levels indicate the presence of a blood brain barrier which may regulate the amount of hor mone entering brain tissues, however the small amounts which undoubtedly do enter the brain may be adequate for the control of cerebral activity

The identification of thyroid hormones or their metabolites in tissues has only been accomplished relatively recently The chromatographic identification of thyroxine in mouse liver[9 0] and of both thyroxine and triiodothyronine in the posterior pituitary[1586 515] have been described More recently, with the use of autoradiography and chromatography, Ford *et al*[514] have demonstrated the presence of thyroxine and triiodo thyronine in almost all tissues of the guinea pig after injection of radio active thyroxine and triiodothyronine Unfortunately large doses of thyroid hormones were employed so that the quantitative significance of this work has yet to be confirmed

Passage of thyroid hormones across cell membranes and their intracellular distribution—The mechanism that determines the entry of thyroid hormones into the cell and factors that control it are unknown From scant evidence at present available it appears that the binding of the hormones to thyroxine binding protein (TBP) of plasma contributes to the regulation of their passage across the cell wall, (this has been discussed in Chapter 4) The importance of TBP was originally postulated as a result of studies on thyroxine binding *in vitro* (see p 54) recently Myant s[1138] experiments on thyroxine binding *in vivo* have given support to the earlier hypothesis

The transfer of thyroid hormones across the placenta has also been investigated in intact animals results of these studies are however sometimes contradictory Although it is generally agreed that thyroxine does not cross from the maternal circulation into the placenta no such agreement has been reached concerning placental concentration of triiodo thyronine[856 649 993 1147 1 42 1140] Although triiodothyronine has been identified chromatographically in foetal plasma after its administration to the mother the possibility exists that the placenta actually concentrates triiodothyronine iodine as iodide and this is subsequently converted to triiodothyronine in the foetal thyroid Waterman and Gorbman[1697] and Geloso[561] have shown that the foetal thyroid does concentrate iodide and convert it to hormonal iodine The very small amounts of thyroxine and

triiodothyronine which cross the placenta would in fact be insufficient for normal skeletal and brain development of the foetus if the foetal thyroid gland were deficient in iodine

Studies on the intracellular localization of thyroid hormones have been hampered by a lack of sensitive analytical techniques so that very large doses of labelled compounds have had to be given in order to detect them in the various cell constituents Thus Lipner *et al* [966] administered as much as 612 µg of L-thyroxine labelled with ^{14}C in the carboxyl group per kg body weight to rats and found an equal distribution of radioactivity in the nuclear mitochondrial and microsomal fractions of the liver However when Lee and Williams[931] gave smaller doses of labelled thyroxine the liver mitochondria were found to concentrate a higher proportion of radioactivity, similar results were obtained by Carr and Riggs[260] who estimated the ^{131}I content of the different fractions while Tabachnick and Bonnycastle[1562] found iodine in almost equal amounts in the particulate and non particulate fractions Although no strikingly selective localization is observed in these subcellular fractions, the distribution has been described as an active process In this respect it is interesting to note that Klemperer[857, 858] observed that the uncoupling of oxidative phosphorylation in liver mitochondria by thyroxine and triiodothyronine *in vitro* is not directly related to the previous mitochondrial "uptake" of the two hormones from the suspending medium

More recently a specific globular protein has been identified in rat skeletal muscle extracts that is capable of binding thyroid hormones[1574] Triiodothyronine is much less firmly bound than thyroxine, this is important when one considers the more rapid disappearance of triiodothyronine *in vivo* from both serum and tissues The cellular thyroxine binding protein fraction has both lower affinity and capacity for binding thyroid hormones than that of serum TBP or albumin this explains the difficulty in identifying cellular TBP in more vascular tissues such as liver Further, when muscle TBP extract is added to crude rat muscle 'deiodinase', deiodinating activity is lost the inhibition being directly proportional to the amount of thyroxine bound to muscle TBP Although the precise physiological significance of this fact is not yet clear this interaction suggests that a role for cellular TBP may be the regulation of the intracellular level of thyroid hormones by influencing their availability to enzymes in the cell

These results do not represent the sole mechanism controlling the entry of thyroid hormones into tissues in fact they only introduce the further questions Do these results represent what actually happens at a cellular level in the intact animal? To what extent would the presence of an intact cell membrane modify the entry and distribution of thyroid hormones in the different subcellular fractions?

(*d*) *Liver Metabolism and Enterohepatic Circulation of Thyroid Hormones*

It was early recognized that the liver concentrated iodine from the blood after administration of very large doses of thyroxine and later released it into the bile[815, 5, 1766, 878] Analysis of bile by biological and chemical methods revealed the presence of unchanged thyroxine and other iodinated compounds[878, 61, 428, 117] The suggestion that liver and bile were involved in the excretion or inactivation of large doses of thyroxine was supported by the later observations that thyroxine produces a greater metabolic stimulation in hepatectomized animals or in animals with ligated bile ducts than in normal animals[841, 605] More recently the importance of the role of liver in the metabolism of thyroid hormones has been inferred from studies on the nature of urinary iodinated metabolites in hepatectomized dogs[498] The reduction of thyroid activity following experimental liver injury in the rat[875] and the elevated plasma PBI in humans with infectious hepatitis further demonstrate the importance of the liver in thyroid hormone metabolism

Very little was known about the fate of the iodine containing compounds of the bile when it is secreted into the intestine until the first experiments with radioactive thyroxine were performed Gross and Leblond[635] showed that a high proportion of radioactivity from a large dose of ^{131}I labelled thyroxine in the rat stomach and jejuno ileum appeared in the bile as much as 75% of the injected ^{131}I was found in the faeces in 24 hr Three years later the same workers[636] again demonstrated the importance of the liver bile and the gastro intestinal tract in thyroxine metabolism using physiological amounts (as low as 0 007 μg thyroxine/rat) of thyroxine labelled both chemically and biosynthetically

The amounts of thyroid hormones concentrated in liver and their entero hepatic circulation vary greatly according to the species of experimental animals used the hormone studied and the dose injected Thus in the rat the laboratory animal most often studied for this purpose Albert and Keating[18] found nearly half the administered radioactivity in the liver 1 min after intravenous injection of ^{131}I labelled thyroxine and more than half of the residual radioactivity in the liver 16 days later The radioactivity appears rapidly in the bile 50–70% of the injected dose being found in the first 24 hr[1580, 1581] Myant[1138] has expressed the biliary clearance rate* of endogenously labelled radioactive thyroid hormone as 2 ml of plasma per hour this is the same as the value obtained when 1 μg of exogenous thyroxine is administered to an adult rat As the liver concentrates more thyroxine when higher doses are administered[636] the clearance rate in the bile increases to a maximum of 35–40 ml of plasma per hr when 2 mg of thyroxine is injected The biliary clearance rate of triiodothyronine reflects

* Biliary clearance rate = [(concentration of ^{131}I in bile)/(concentration of ^{131}I in plasma)] × (rate of flow of bile in ml/hr)

its higher and more rapid concentration in the liver (see Fig 2) This varies from 30 to 70 ml per hr but is independent of the injected dose between 1 μg and 2 mg of triiodothyronine[1138] This probably results from the relatively loose binding of triiodothyronine to serum proteins even when it is administered in small amounts The biliary excretion of the two hormones also varies directly with the physiological state of the animal Thus Klitgaard[862] observed that the radiothyroxine present in bile and the volume of bile flow were simultaneously and markedly raised *in hyperthyroid rats and lowered in hypothyroid or surgically thyroidecto* mized animals The biliary excretion rate could however be increased in hypothyroid animals by the injection of larger amounts of these hormones[1343] After injection of labelled 3 3'-diiodo L thyronine a smaller amount of ^{131}I was found in the liver than after either thyroxine or triiodo thyronine, though the rate of ^{131}I secretion in the bile was greatly increased 3 3 5'-Triiodo L-thyronine and 3 5 3 triiodothyroacetic acid were excreted at the same rate as thyroxine[1349 1350 1348] The differences in the rate of biliary excretion of the different iodothyronines and triiodothyro acetic acid in the thyroidectomized rat are compared in Fig 19 The

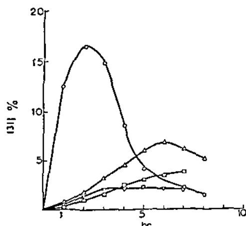


Fig 19

Total radioactivity (percentage of dose) secreted in the bile of thyroidectomized rats at different time intervals after the administration of four iodothyronines labelled with ^{131}I □—□ thyroxine △—△ 3 5 3 triiodothyronine ▽—▽ 3 3 5 triiodothyronine ○—○ 3 3 diiodothyronine (From Roche 1957)

sharp fall in radioactivity in bile of animals injected with 3 3' diiodothyronine after the first 2–3 hr is explained by the more rapid destruction and elimination of this compound[1524]

Although it has been known for some time that thyroxine injected into a ligated intestinal loop is rapidly absorbed[1421], proof of a recycling or

enterohepatic circulation of thyroxine was only conclusively obtained relatively recently from the work of Albert and Keating[18] They found that when equilibrium was attained the gastrointestinal tract in the rat contained nearly half of the ^{131}I labelled thyroxine administered (or its metabolites) The rapidity of recirculation and extent of intestinal absorption of radioactivity principally via the portal and lymphatic drainage were emphasized by the disparity between the rate of ^{131}I secretion into the bowel which exceeded 100% of the injected dose per hr and the rate at which it left the bowel into the faeces which was only about 3% per hr More recently the intestinal reabsorption of circulating endogenously labelled hormone has been estimated to be about 60% of the biliary ^{131}I from a calculation of the biliary and faecal clearance rates[1139] With isolated portions of the gastro intestinal tract, however only 20–25% of labelled thyroxine were found to be absorbed [3] Briggs *et al* [216] infused bile containing radioactive thyroxine into the duodenum and also observed a similar absorption of radioactivity The efficiency of duodeno intestinal absorption is about the same for both hormones in dogs[94, 95, 93]

The importance of this enterohepatic circulation of thyroid hormones in the rat is not reflected in man, all pathways of elimination of hormonal iodine in the two species are radically different, as will be seen later Although the human liver very rapidly concentrates thyroxine in large amounts[839, 1142, 1239, 1633] the rate of excretion of thyroxine iodine in bile is much less than in the rat[615] In Myant s[1136] studies the biliary clearance rate of thyroxine was estimated to be between 200 and 600 ml of plasma per day This slow biliary excretion rate is accompanied by a poor gastro intestinal reabsorption of the hormones since only about 30% of bile iodine is reabsorbed in man The total amount of iodine lost by this pathway in man is still not large compared to that in the rat since the faecal clearance of plasma hormone represents less than 5% of the extra thyroidal organic iodine pool[156] Animals of other species studied namely the cat dog and sheep seem to fall between the rat and man as regards the magnitude of enterohepatic circulation[1579, 496, 285]

For a long time the iodine compounds in the bile were grouped as unchanged thyroxine or inorganic iodide which were present in varying proportions[678, 428, 117, 285] With the introduction of chromatographic analysis it has been shown that although some unchanged thyroxine is present in the bile, a large part of biliary iodine is represented neither by thyroxine nor inorganic iodide With the aid of radioautography of chromatograms Taurog *et al* [1580, 1581] first demonstrated that much of the injected thyroxine in the rat was conjugated in the liver with glucuronic acid and secreted as such into the bile The glucuronides of thyroxine and triiodothyronine have since been detected in the bile of rat dog rabbit sheep man and in rat liver[1581, 90, 1361, 863, 215, 1579, 1415, 1137]

[148, 498] The glucuronide of thyroxine has also been enzymically synthesized *in vitro*[80]. A more complex picture is, however, seen in the bile after administration of thyroxine or triiodothyronine. Besides the unchanged hormones, their glucuronides and some inorganic iodide, Roche *et al.*[1361] observed several other iodinated compounds in rat bile, seen on the chromatograms in Fig. 20. A picture of similar complexity has been observed in the bile of dogs after administration of triiodothyronine[494, 498]. The radioactive compounds marked II and B in Fig. 20 have been tentatively identified as the pyruvic acid analogues of thyroxine and triiodothyronine; the identity of the other compounds is not known. However, the more important derivatives in the bile are the glucuronides of the hormones; this is especially noticeable when the hormones are administered in physiological amounts. This is also true for the formation of glucuronides of 3:3'-diiodothyronine and 3:3':5'-triiodothyronine[1349, 1350]. In all animals, iodide accounts for only a small fraction of bile iodine; this does not, however, indicate any lack of deiodinating capacity in the liver: the liver is in fact the principal deiodinating organ in the body (see Chapter 8). It merely reflects the very rapid removal of iodide into the blood stream and thence to the thyroid gland and kidney. Chromatographic analysis has clearly disproved the earlier beliefs which were based on solubility properties, that most of the non-thyroxine iodine in bile is iodide. In the rat, the only derivative of thyroxine found in the bile after injection of ^{131}I was the glucuronide[1581]. It is not possible to conclude from chromatographic analysis in the absence of specific radioactivity determinations what proportion of the hormones are present as glucuronides in the steady state. The fact that about 60–70% of ^{131}I appears in the faeces of rats after a duodenal infusion of bile containing radioactive glucuronide of thyroxine and the observation that the glucuronide is less readily absorbed than is free thyroxine in the intestinal tract[216] indicates that glucuronide formation is a process of detoxication. Glucuronide formation also plays a part in regulating the level of circulating hormone since it releases the hormone for reabsorption after hydrolysis by the β-glucuronidase in the large intestine[216, 1137]. The capacity of the liver to form glucuronides of thyroid hormones is limited, although quantitative differences are found with different compounds. These differences[1343, 1579] are difficult to interpret since the daily thyroid secretion rate is only known for L-thyroxine.

(e) *Excretion of Thyroid Hormones*

The large species differences in the enterohepatic circulation of thyroid hormones are reflected in their urinary and faecal excretion. Thus in the rat, which has a very intense enterohepatic circulation, the main excretory pathway after large doses of thyroxine (about 75%) is in the faeces[835],

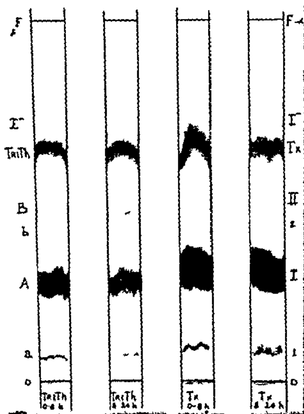
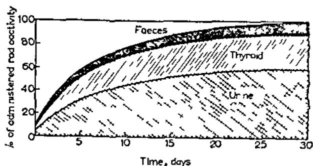


Fig 20

Autoradiogram of chromatograms of whole rat bile pooled at 8 and 24 hr after the administration of I labelled L thyroxine and L triiodothyronine showing the large number of iodinated metabolites secreted in the bile Tx = thyroxine TRITh = triiodothyronine I and A = glucuronides of thyroxine and triiodothyronine respectively II and B are tentatively identified as the corresponding tetraiodo and triiodothyropyruvic acids I⁻ = iodide other metabolites are unidentified Solvent Collidine—H_2O—NH ascending (From Tata *)

with smaller doses of thyroxine, 50–70% of the injected material was eliminated in the faeces in 3 days[635 814 18] At the other end of the scale i e in man, the faecal excretion is very low and the major pathway of excretion of thyroid hormones is in the urine [0 16 1142] This difference in the elimination of thyroxine and its metabolites in man and rat is seen in Fig 21



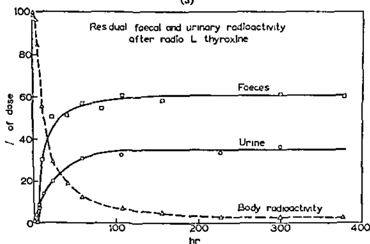


Fig 21

Species difference in the pattern of excretion of radioactivity after the administration of ¹ I labelled L thyroxine to man and rat

(a) In man the excretion of thyroxine iodine in the urine is greater than the faecal excretion

(b) In the rat the faecal excretion is predominant (From (a) Ingbar and Freinkel [8] and (b) Albert and Keating)

A potential source of error in the interpretation of these experiments is contamination of the labelled thyroxine by iodide if a sample of labelled thyroxine contained 10% of its radioactivity as iodide then about 30% of urinary ^{131}I at the end of 6 hr would be due to the contaminant since

iodide is excreted much faster than thyroxine It is probably for this reason that urinary excretion values are found to vary from one experiment to another However errors from this source have not invalidated the finding of species differences mentioned above

The faecal clearance rate of endogenous thyroid hormone in the rat is about 19 5 ml plasma per day as compared to a biliary clearance rate of 44 6 ml per day In spite of the intestinal reabsorption of biliary iodinated compounds this faecal loss of iodine represents about 25% of the daily thyroxine secreted by the rat s thyroid gland Thyroxine itself is probably the only iodinated faecal component (see page 144) so it would appear that in this animal the thyroid gland secretes more hormone than the body requires In man on the other hand, the faecal clearance rate of chemically or biologically synthesized thyroxine is only about 200–450 ml plasma per day or less than a twentieth of the total extrathyroidal organic iodine pool [156, 1634] The higher faecal excretion values of 21–32% of L thyroxine ^{131}I in man reported by Johnson and Beierwaltes[615] are very likely due to incomplete absorption of thyroxine after oral administration The urinary clearance rate of thyroxine in man is about twice the faecal clearance rate [158, 212, 89, 1634] Since urinary iodine is mostly inorganic (see page 144) the clearance rate has to be measured either by preventing reutilization of this iodide by blocking the thyroid gland with antithyroid drugs or by determining the thyroidal ^{131}I/urinary ^{131}I accumulation ratio Excretion of thyroid hormones in other species has not been studied in such detail although information is available on the rates of faecal and urinary excretion in the dog, cat mouse and rabbit [85, 632, 236, 94, 1633] In general, faecal elimination exceeds urinary excretion in rodents whereas in other mammals especially in man the principal route is via the kidney

An interesting suggestion has recently been put forward to account for these species differences, based on the relative faecal volume in different species[1633] Faecal elimination of thyroxine is roughly proportional to average faecal volume per gramme of body weight of each species, this is shown in Fig 22. Thus the higher the faecal volume the greater is the loss of thyroxine in the faeces The faecal volume depends on the relative lengths of the intestine and the rates of flow of faecal contents the amount of thyroxine lost depends on the rate of hydrolysis of its glucuronide and rate of reabsorption of free hormone in the intestine These relationships are further complicated by the fact that faecal volume is itself raised by acute or chronic increases in level of circulating thyroid hormones this is seen in faecal volumes in hypothyroid and normal rabbits in Fig 9, and also in other experiments[1008, 1175] A relationship between the faecal volume and the rate of thyroxine excretion has also been derived from experiments in which the faecal volume of rats was altered by changes in the diet[1673]

A more rapid rate of excretion of triiodothyronine than of thyroxine

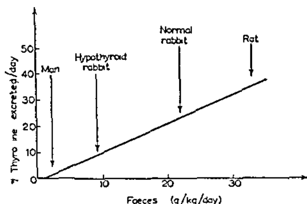


Fig 22
Relationship between the average faecal excretion of thyroxine and the faecal volume in different species of mammals (From Triantaphyllidis[1633])

has been demonstrated in man rat and dog[1639, 840, 498] but besides an increased overall rate of excretion, the main pathway of excretion of triiodothyronine is different In the rat about 55% of total ^{131}I appeared in the urine after injection of labelled triiodothyronine compared with about 36% after labelled thyroxine under similar conditions This is all the more *striking since the rate of enterohepatic circulation of triiodothyronine is* the same or even higher than that of thyroxine in the rat[840, 1343] In man even less triiodothyronine iodine is excreted via the faeces than thyroxine iodine Since neither the triiodothyronine pool nor the rate of thyroidal secretion of this hormone is known it is impossible to ascribe absolute figures to the physiological rates of its clearance from the plasma into the faeces and urine It is probable that the higher biological activity of triiodothyronine is related to its higher rate of metabolism and excretion, these in turn are related to the weaker binding of triiodothyronine to plasma and tissue proteins The observation that thyroxine and triiodothyronine show equal biological potencies in birds[1452, 1151] supports this concept since Tata and Shellabarger (unpublished) have found that disappearance rates of thyroxine and triiodothyronine are identical in fowl It does not however necessarily follow that a higher excretion rate of an iodothyronine means an enhanced biological potency Thus 3 3 diiodothyronine which is degraded and excreted much faster than triiodothyronine has relatively lower biological activity while the rate of excretion of the biologically inactive 3 3 5 triiodothyronine is about the same as that of thyroxine[1349, 1524] About 85–90% of 3 3 diiodothyronine iodine is excreted in the urine of both rat and man its urinary excretion in humans is so rapid that Stanbury and Morris[1524] doubt whether this compound can be detected in the blood even if it were secreted by the thyroid gland at the same rate as is thyroxine

Nature of excretion products of thyroid hormones—Differences in the routes of excretion of iodothyronines in various species are further reflected in differences in the iodinated substances excreted Analysis of urinary and faecal iodine shows that the thyroid hormones follow two totally different metabolic pathways Paper chromatographic analysis of urine and faeces have confirmed the earlier observations that whereas a large proportion of faecal iodine is present as free thyroxine in urine it is present as iodide[1360, 1579, 4, 9, 350, 635, 638, 16, 0] Most of the thyroxine appearing in the faeces is derived from its glucuronide after hydrolysis by intestinal bacteria In general less than 10% of urinary iodine represents organic compounds but this figure may be higher if large doses of the hormones are administered In some cases small amounts of unchanged thyroxine have been detected in the urine but in most of these experiments either thyroxine was not administered at physiological levels or the normal metabolic pathway was altered by hepatectomy or bile obstruction[1091, 819, 820, 1259, 151, 152, 498, 497] A small amount of free triiodothyronine has also been detected in the urine of dogs[498]

Some investigators have reported the appearance of diiodotyrosine in the urine as a metabolite of thyroxine[819, 8 0, 1259, 151, 15, 0 16] In one case as much as 68% of thyroxine was claimed to be converted to diiodo tyrosine A substantial conversion of thyroxine to urinary diiodotyrosine is incompatible with the fact that diiodotyrosine is so much more rapidly degraded in the body than thyroxine and that less than 10% of it is excreted unchanged[9, 9, 17, 814] However the methods used to identify diiodotyrosine were based on solubility properties on a chromatographic separation in phenol water which gives poor resolution of the known iodo amino acids When more suitable solvents were used (butanol acetic acid butanol dioxan ammonia *tert* amyl alcohol ammonia) the apparent 'diiodotyrosine in rat urine was shown to consist of the pyruvic acid analogues of thyroxine and triiodothyronine These metabolites are also present in the bile (substances labelled B and II in Fig 20) and their urinary concentration increases upon ligature of the bile duct Similar results were obtained by Flock *et al*[498] in dogs Fletcher[491, 492] believes that the diiodotyrosine found in human urine is derived from circulating thyroglobulin released after therapeutic doses of radioactive iodine thus Horst and Heuwieser[761] have detected all the constituent iodinated amino acids of thyroglobulin in the urine of patients treated with radioactive iodine for thyroid carcinoma and other disorders Small amounts of glucuronides of thyroxine and triiodothyronine have been observed in the urine of rat and dog[1578, 496] On the whole the levels of organic iodine compounds (such as free thyroid hormones, their glucuronides and pyruvic acid analogues) in the urine are markedly increased when the enterohepatic

circulation of thyroid hormones is interrupted by ligature of the bile duct or total hepatectomy[1363, 498, 496, 497]

Before summarizing it should be mentioned that none of the fractionations of urinary iodine described above was carried out with endogenously labelled thyroid hormone and thyroglobulin was often present in the circulation The amounts of organic iodine compounds in the urine increase when higher doses of hormones are administered hence it is not yet certain to what extent organic iodine compounds are excreted in the urine of normal subjects It is however clear that whereas urinary excretion reflects a deiodinating metabolism of thyroid hormones, faecal *excretion is the end point of their enterohepatic metabolism after they have* undergone a process of conjugation with glucuronic acid and hydrolysis

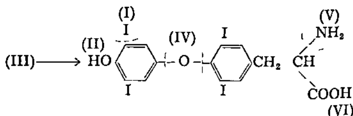
Factors that affect the excretion of thyroid hormones—The same factors that affect the overall metabolism of thyroid hormones are involved in their excretion[882, 156, 1663, 236, 1633] In any study of the excretion rate the dose of hormone *administered is important because the route of excretion of* excessive doses of the hormones will vary according to the species studied Thus, most of an excessive dose of thyroxine or triiodothyronine will appear in the urine of man while in the rat only the faecal excretion of iodine will be accelerated The effect of the iodine level in the diet of the rabbit on the biological half life of thyroid hormones has already been mentioned (page 131) Another nutritional factor has been recently studied Van Middlesworth[1673] found that a diet of soya flour or laboratory chow increased the faecal excretion rates of thyroxine in the rat two to twenty fold compared with those in animals on other diets this was accompanied by a lowering of the level of circulating hormone (PBI) From these results Van Middlesworth suggests that some diets increase the iodide requirement of rats this may account for the production of goitre which has been termed as thyroxine depletion goitre These disturbances in excretion are themselves of interest their aetiology can only be established by more complete iodine balance studies[1404, 1675, 1102, 1466]

Finally the apparent rates of excretion of thyroxine and triiodothyronine vary according to the positions of the iodine label Thus thyroxine labelled in the phenolic ring (3 and 5 positions) is found to be excreted at a rate 50% higher than thyroxine labelled in 3 and 5 positions[1360] further the lower rate of excretion of the latter was accompanied by an increase of the *ratio of urinary organic to inorganic iodine which was 100% greater than* that found in rats injected with 3 and 5 labelled thyroxine This greater biological lability of iodine in the phenolic ring is also seen with 3 5 3 -triiodothyronine[1339] moreover modification of the alanine side chain does not influence the lability of iodine atoms in the diphenyl ether moiety this is seen in the excretion rates of tetra and tri iodothyroacetic acids after two different methods of labelling[1348, 1719]

CHAPTER 8

BIOCHEMICAL PATHWAYS OF THYROID HORMONE METABOLISM

BIOCHEMICAL transformations of the thyroid hormone molecules in the body tissues can occur at various points on the molecule the six reactions which will be considered below are (I) deiodination (II) oxidation of the phenol, (III) conjugation of the phenol, (IV) rupture of the diphenyl ether linkage (V) oxidative deamination of the alanine side chain, and (VI) decarboxylation Possible metabolic pathways for



the principal iodothyronines are summarized in Table VIII the more important aspects relating to their physiological significance are reviewed separately

Labelled thyroid hormones of high specific activity have only so far been obtained with ^{131}I as the label once this is lost the fate of the remainder of the molecule is unknown For this reason deiodination of thyroxine and allied compounds has been investigated more thoroughly than the other reactions

(I) DEIODINATION

Ever since the demonstration by Blum and Gruntzer[184] of the break down of thyroglobulin to iodide deiodination has been considered the most important reaction in the metabolism of thyroid hormones In all

TABLE VIII

Summary of metabolic pathways of iodothyronines

Pathway	In vivo / in vitro	Iodothyronine	Metabolites identified	Principal organs, tissues or enzymes involved	Metabolites found in
(I) Deiodination	In vivo	Thyroxine 3,5,3'-Triiodothyronine 3,3'-Diiodothyronine 3,3',5'-Triiodothyronine	Iodide, traces of partially deiodinated iodothyronines	Liver and kidney, other tissues to lesser extent	Urine; in small amounts in most tissues
	In vitro	Thyroxine 3,5,3'-Triiodothyronine	Iodide, traces of triiodothyronine from thyroxine	Liver, kidney, skeletal muscle, brain	—
(II) Oxidation of phenol	In vitro	Triiodothyronine 3,3'-Diiodothyronine 3-Monoiodothyronine	Quinones, semiquinones and hydroxylated compounds	Mushroom polyphenol oxidase, Harding Passey melanoma	—
(III) Conjugation of the phenol (1) With glucuronic acid	In vivo	Thyroxine 3,5,3'-Triiodothyronine 3,3'-Diiodothyronine 3,3',5'-Triiodothyronine	Glucuronides of iodothyronines	Liver, kidney	*Bile, small amounts in urine*
	In vitro	Thyroxine	Glucuronide of thyroxine	Liver slices, uridine diphosphoglucuronic acid + liver microsomal activating enzyme	
(2) With sulphate	In vivo	Triiodothyronine	Sulphate ester	Liver	
(IV) Rupture of diphenyl ether bond	In vivo	Thyroxine	Diiodotyrosine	Not identified	Urine
	In vitro	3,3'-Diiodothyronine	Monoiodotyrosine	Mushroom polyphenol oxidase	
(V) Deamination	In vivo	Thyroxine 3,5,3'-Triiodothyronine	Acetic and pyruvic acid analogues of thyroxine and triiodothyronine	Liver, kidney	Bile, kidney, liver, urine
	In vitro	Thyroxine 3,5,3'-Triiodothyronine	Acetic acid analogues	Kidney and brain homogenates, kidney mitochondrial extracts	
(VI) Decarboxylation	In vitro	Thyroxine	Thyroxamine (?)	Intestinal m[illegible]	

animals urinary iodide is the most abundant degradation product of organic iodine compounds The liberation of iodide as a thyroid hormone metabolite plays an important part in the iodine economy of the animal since it is recirculated to the thyroid and reincorporated into thyroglobulin furthermore this process of successive thyroidal iodination and peripheral deiodination must repeat itself many times since the kidney reabsorbs about 97% of the filtered iodide Even in rodents, where faecal excretion of iodine is relatively high this reutilization of iodide must play an important part

All organs and extravascular tissues appear to possess some ability to deiodinate the thyroid hormones Thus in the guinea pig, most tissues which do not concentrate iodide have a higher radioactive iodide concentration than that of the serum after the injection of ^{131}I labelled thyroxine and triiodothyronine[514] This would reflect a breakdown of the hormone after its concentration by the tissue As we have already seen triiodothyronine is deiodinated faster and to a greater extent than is thyroxine in the organs of intact animals which results in an increased urinary iodide excretion after triiodothyronine administration The liver plays an important part in the process of deiodination and excretion of urinary iodide is markedly depressed in hepatectomized animals[498] and in humans suffering from cirrhosis of the liver[1398] The kidney is also a powerful deiodinating organ this has been shown in studies on rabbit kidney perfused with labelled triiodothyronine[587] Urinary iodide excretion chiefly represents iodine from the extrathyroidal breakdown of thyroid hormones and the same factors that affect urinary excretion (i e cold thyroid status etc) will similarly affect deiodination of the hormones in the tissues

The first studies on deiodination of thyroid hormones by isolated tissues gave negative results Roche and co workers found that the iodotyrosine deiodinase present in the thyroid liver kidney and heart slices or extracts had no action on thyroxine[1365, 1342] Later however reports appeared on the deiodination *in vitro* of thyroxine and triiodothyronine during incubation with tissue preparations[1005, 25] of the various tissues studied liver had the highest deiodinating activity These studies have been followed by others reporting deiodination of the thyroid hormones *in vitro*[1512, 911, 1398, 149, 1578, 157, 1003, 1399, 1146] There is however no agreement in these reports regarding the modalities of deiodination and it would be profitable to consider here the different techniques used and the interpretation of the results in order to differentiate between enzymic and non enzymic deiodination

In the first place deiodination of labelled compounds can only be said to have occurred if the presence of iodide is established by unequivocal methods namely chromatographic identification in more than one solvent

system in the presence of carrier iodide extraction into carbon disulphide after oxidation with ferric ions, electrophoretic mobility, isotopic dilution to constant specific activity and adsorption on suitable ion exchange resins False conclusions will be drawn if non specific tests such as water solubility are used for instance when ^{131}I labelled thyroxine or triiodothyronine are incubated with liver or kidney preparations they are largely converted to the glucuronides These are water soluble compounds and are not pre cipitated by protein precipitants this has led certain workers to infer the presence of iodide, when in fact iodide may have represented only a small part of non hormonal iodine[1512 1003]

The great variability in the R_F values of iodotyrosines iodothyronines and iodide in the solvents generally used is well known and is due to such factors as temperature and loss of volatile constituents in the solvent For this reason when chromatographic analysis is used for the identification of thyroid hormone metabolites it is essential to use more than one solvent system and to locate the metabolites with carriers For example Larson *et al* [911] have identified triiodothyronine as the sole metabolite of thyroxine after incubation with kidney slices the identification depended on one dimensional chromatography in butanol dioxan ammonia later this group[16 7] found that tetraiodothyroacetic acid was formed from thyroxine by kidney mitochondrial extracts again butanol dioxan ammonia was used Now triiodothyronine and tetraiodothyroacetic acid do not separate in this solvent system so that neither thyroxine metabolite can be said to have been characterized by this method however tetraiodothyroacetic acid was characterized by other means and it is likely that the apparent triiodothyronine from thyroxine was not triiodothyronine at all Moreover the formation of triiodothyronine from thyroxine involves the liberation of iodide and none was demonstrated lastly the fact that triiodothyronine itself is highly susceptible to deiodination makes it unlikely that it will be found as the principal metabolite of thyroxine

In the many reports on deiodination of thyroid hormones *in vitro* sufficient evidence has not been given to establish that the reaction has been enzyme catalysed Thyroxine and triiodothyronine are known to undergo spontaneous deiodination in solution at a very rapid rate in some circumstances Lissitzky *et al* [976] in fact believe that the deiodinase reaction of Sprott and Maclagan[1512] is likely to be entirely non enzymic because deiodination occurred equally in the boiled and unboiled preparations Recently an apparent and reversible deiodination of thyroxine and other iodophenols has been observed by Tata (unpublished) this occurs when the iodophenol passes from an organic to an aqueous solvent resulting in dissociation of the phenolic group This spontaneous reaction can be pre vented by the thyroxine binding proteins and addition of these proteins to the reaction mixture will cause reversal of the reaction and the reappearance

of thyroxine No reversal of deiodination takes place when it has been effected enzymically The addition of serum or thyroxine binding proteins to a thyroxine deiodinase system at different time intervals during the reaction has shown that in fact both enzymic and non enzymic reactions are proceeding simultaneously it has by this means been found possible to measure the true rate of the enzymic reaction All the methods for the identification of iodide discussed above have been used in the study of deiodination by brain and muscle preparations[1578 1572 1573]

A dissimilarity in the rates of deiodination of thyroxine and triiodo thyronine *in vivo* and *in vitro* has been observed, and may at first seem surprising Whereas triiodothyronine is deiodinated much faster than thyroxine in the intact animal (at least two to three times on the basis of urinary excretion and thyroid reabsorption) in isolated tissue preparations the rates are almost identical or if anything a little slower for triiodo thyronine[1578 1572 1573]

No explanation has been found for this disparity it is most unlikely that different enzymes are involved in the deiodination of the two hor mones The difference could however be explained on the basis of a more rapid diffusion of triiodothyronine into tissues *in vivo* because of its weaker binding by the thyroxine binding protein of serum (see page 54) If this were the case the slight difference in the affinity of the deiodinating enzyme for thyroxine and triiodothyronine would not be the rate limiting factor in the intact animal

Tong *et al*[16 9] have observed differences in enzymic deiodination of diiodotyrosine (which is however, not secreted by the normal thyroid gland) in different tissues, the deiodinase described by Roche and co workers was present in the liver and could deiodinate both diiodo tyrosine and its lactic acid analogue 3 5 diiodo *p* hydroxyphenyllactic acid whereas the enzyme from the thyroid could only deiodinate the amino acid Whether two different enzymes are involved in the deiodi nation of iodotyrosines and iodothyronines has yet to be shown, but a pathway of deiodination in brain preparations has been suggested in which the thyroid hormones would first undergo oxidative deamination This was based on the accumulation of significant amounts of the acetic acid analogues of thyroxine and triiodothyronine after deiodinase activity was blocked by mercuric chloride reactivation of the deiodinase with hydrogen sulphide caused the disappearance of these acetic acids

The failure to detect the acetic acid analogues or any other derivatives of the thyroid hormones when the latter are incubated with skeletal muscle preparations shows that direct deiodination of the iodothyronines is the only metabolic process occurring in this tissue[1572] The situation is more complex in liver and kidney here simultaneous conversion of thyroxine and triiodothyronine to many other iodinated compounds takes place

Moreover most of these metabolites have not yet been identified For this reason, skeletal muscle is to be preferred for quantitative studies on the distribution, purification and properties of iodothyronine deiodinase although its potency is lower than that of the liver and kidney enzyme[1573] Disagreement on the intracellular distribution of the deiodinases is probably due to the different methods used for determining deiodination [711, 712, 713, 1003, 1573, 1518, 1399]

Progress in purification of deiodinase' has been slow and little information is available as to its nature and mode of action The crude enzyme from muscle is very heat labile and is denatured easily by successive freezing and thawing in solution it loses activity rapidly on storage at 0 C it is also affected by mercurial compounds and other inhibitors of thiol enzymes The deiodinase which appears to be iron- and flavine-dependent, can only exert its action on free (non protein bound) thyroxine This was demonstrated when non competitive inhibition of enzyme activity by certain cellular or plasma proteins was obtained inhibition was directly proportional to the amount of substrate which was protein bound This inhibition of iodothyronine deiodinase activity may account for the failure of workers to observe deiodination of thyroxine and triiodothyronine *in vitro*[1355, 976] This inhibition will be especially marked in vascular tissues such as liver, thyroid and kidney the same tissues can however deiodinate the iodotyrosines since the latter are only very feebly bound to plasma proteins

Interest in the biological deiodination of thyroxine arose from the discovery[641, 642, 644, 646] that triiodothyronine possesses about five times the physiological activity of thyroxine It has been repeatedly suggested that thyroxine exerts its peripheral action only after its partial deiodination to triiodothyronine evidence for such a deiodination was found in the apparent formation of triiodothyronine from thyroxine in thyroidectomized mice[637], but the claim of Pitt Rivers *et al* [1231] that this reaction occurs in athyreotic humans has not been confirmed (Lassiter and Stanbury personal communication)

Indirect evidence suggesting that thyroxine must be converted to triiodothyronine in order to exert its biological action was based on the effect of the antithyroxine drug *n* butyl 4 hydroxy 3 5 diiodobenzoate (BHDB) on the overall metabolism of the two hormones BHDB was found to diminish the output of urinary iodide when thyroxine was injected into rats but did not do so when triiodothyronine was injected[1009, 1720, 1664] From this work Maclagan and Wilkinson have interpreted the prevention of deiodination of thyroxine to triiodothyronine as the antithyroxine mode of action of BHDB The findings of Roche *et al* [1333] however are different these workers administered thyroxine labelled in the 3 5 3 5 and 3 5 3 5 positions to thyroidectomized rats and found that BHDB

enormously enhanced urinary ^{131}I when administered with the 3 5 labelled thyroxine They concluded that the antithyroxine drug acts by potentiating the deiodination of thyroxine, rather than by direct inhibition in peripheral tissues

Subsequent experiments have shown that small amounts of triiodo thyronine are undoubtedly produced from thyroxine in various tissues other than the thyroid gland[829, 514, 1578], but it is not known how far this is a major source of extrathyroidal triiodothyronine The conclusion that triiodothyronine is derived from thyroxine and is the peripherally active compound has to be weighed against the fact that the thyroid secretes triiodothyronine at a not inconsiderable rate[146, 159]

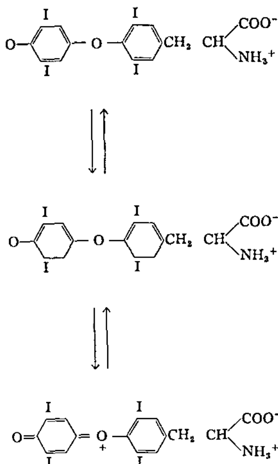
Furthermore no significant amounts of triiodothyronine have been detected after injection of labelled thyroxine into animals Specific radio activity measurements are essential for the assessment of the quantitative importance of thyroidal triiodothyronine on the one hand and extra thyroidal triiodothyronine on the other

Studies on deiodination have been extended to triiodothyronine and it has been shown[1362, 1351] that rat kidney and skeletal muscle can convert this hormone to 3 3 diiodothyronine It is unlikely however that much triiodothyronine would be thus metabolized, since the iodine atom in the 3 position is more labile than the iodine atoms in the 3 and 5 positions[1339] A complicating factor appears in the finding[1367] that partial hydrogenation of thyroxine can give rise to 3 3 5 triiodothyronine if this is so then the chemical and biochemical labilities of the iodine atoms in thyroxine must be different

Whether the deiodination of thyroid hormones is directly related to their physiological activity has yet to be discovered It is however of interest that their deiodination is more rapid in hyperthyroid humans and animals than in hypothyroid subjects[1305, 156, 789, 1633] It is unlikely that deiodi nation in hyperthyroid animals represents a detoxication mechanism for removal of excess hormone since a large part of inorganic iodide thus released would be reabsorbed by the thyroid gland biliary and faecal excretion would be more efficient ways of disposing of excess of hormone Some regard this as a measure of iodine economy in the body but Tata s (unpublished) recent demonstration that salt water fishes possess as active a deiodinase as fresh water fishes indicates that this is a ubiquitous reaction which occurs irrespective of the concentration of environmental iodine

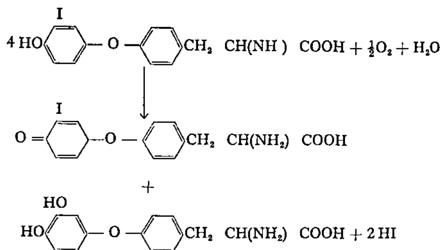
(II) Phenolic Oxidation and Hydroxylation

Niemann put forward the hypothesis, more than 17 years ago that the physiological activity of thyroxine is governed by an equilibrium state between the phenolic and quinonoid forms of the molecule with a possible semiquinone intermediate[1155, 1154, 1153]



This hypothesis was based on the fact that *ortho* thyroxine which could also form a semiquinone had physiological activity while *meta* thyroxine had none No experimental evidence was put forward to support the theory

More recently Lissitzky and his colleagues have shown that thyronine *and some of its halogenated derivatives can be oxidized (like tyrosine) by* the ascorbic acid Fe^{++} O_2 system and by the tyrosinase from mushrooms [974, 971, 972, 973, 977] The iodinated substrates oxidized were 3 monoiodo thyronine 3 monoiodothyronine 3 3 diiodothyronine 3 5 3 triiodo thyronine and 3 5 3 triiodothyroacetic acid in decreasing order Oxida tion was often accompanied by deiodination Thus the oxidation of 3 monoiodothyronine gives rise to the semiquinone form of 3 iodo-thyronine 3 hydroxythyronine and an equivalent amount of iodide



Thyroxine itself is not oxidized by either the ascorbic acid Fe^{++} O_2 system or the mushroom tyrosinase Further no polyphenol oxidase activity has been found in animal tissues with the exception of the Harding Passey melanoma[975] For these reasons it is unlikely that these reactions represent models of thyroid hormone metabolism, nevertheless they form an interesting series of diphenyl ether oxidations *in vitro*

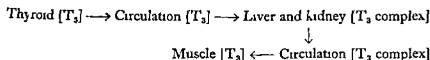
(III) Conjugation of the Phenolic Group

The phenolic group in the iodothyronines can undergo conjugation *in vivo* as can other phenolic compounds These lead to the formation of (1) glucuronides and (2) sulphate esters, glucuronide formation is the more important reaction in the iodothyronine series and has been more extensively studied than sulphate esterification it occurs principally in the liver and the conjugates of thyroxine and triiodothyronine have been found in the bile Other tissues especially kidney are capable of forming thyroid hormone conjugates both *in vivo* and *in vitro*[920 1579 498] The glucuronides are not however found in faeces since the intestinal mucosa contains a β glucuronidase Glucuronides of D thyroxine D 3 5 3 triiodothyronine and 3 5 3 triiodothyroacetic acid have also been detected in bile[1364 1348 1080] In normal animals the glucuronides of thyroxine and triiodothyronine are rarely found except in bile though it has been shown that large amounts of these compounds can pass into the bloodstream during biliary obstruction i e after experimental bile duct ligature in rats[1363] or in patients with obstructive jaundice this accounts for the high blood organic iodine levels and correspondingly low thyroid ^{131}I uptake in such patients[1415 148 1677] It has also been shown that glucuronide formation does not take place in patients with infectious hepatitis or in rats that have been poisoned with allyl formate[1677]

Evidence that conjugation with β glucuronic acid occurs on the phenolic group is obtained from the following considerations (1) ninhydrin treatment of the glucuronide of thyroxine labelled with ^{14}C in the carboxyl group releases quantitatively $^{14}CO_2$[583] (2) glucuronides of both thyroxine and triiodothyronine give positive ninhydrin reactions (α amino acids) but do not react with diazotized sulphanilic acid[1383], (3) enzymic synthesis of thyroxine glucuronide under conditions which would lead to the formation of a glucuronide ester has been achieved[802]

One further reaction of hydroxyaromatic compounds should be considered briefly methylation Methylation of phenols has only recently been studied (see *Nutrition Reviews*, 1957) and so far no methyl ether of the iodothyronines has been detected in biological material However Maclagan and Wilkinson have shown that the administration of the drug *n* butyl 4-hydroxy 3 5 diiodobenzoate to humans is followed by the appearance of 3 5 diiodo 4-methoxybenzoic acid in the urine Methyl ethers of various phenolic drugs have also been demonstrated in different animals[357] However methylation does not appear to have any physiological importance in the metabolism of naturally occurring aromatic amino acids

The first attempts to identify the sulphate esters of thyroxine and triiodothyronine in the bile after administration of ^{131}I-labelled hormones and ^{35}S labelled cystine gave no positive results[1361] More recently Roche and co workers[1347, 1354] have succeeded in identifying by chromatography the sulphate esters of triiodothyronine and triiodothyroacetic acid in bile after administration of the free acids to rats Little more is known of these esters Roche *et al* [1363] and Gross *et al* [634] have described a new compound of unknown structure obtained after injection of ^{131}I labelled triiodothyronine in the rat and rabbit this material has chemical properties which suggest that it may be a sulphate ester (lability in acid solution and chromatographic behaviour) It is found in bile blood and kidney perfusates but not in muscle Gross and his colleagues[634] suggest that this complex is the transport form of triiodothyronine which is synthesized by the liver and kidney thus triiodothyronine (T_3) from the thyroid gland would undergo the following reactions in order to enter the peripheral target cells



Since triiodothyronine only represents a small fraction of the thyroid hormone synthesized and secreted into the circulation it is not possible to say at present how far this transport mechanism really represents the physiological process of hormone distribution

(IV) Rupture of the Diphenyl Ether Linkage

Biological rupture of the diphenyl ether linkage of thyroxine would be the converse of the reaction postulated for its biosynthesis which, as we have seen entails the condensation of two molecules of diiodotyrosine with the loss of one alanine side chain Many reports have appeared in the literature claiming the detection of diiodotyrosine in urine after administration of ^{131}I or ^{131}I labelled thyroxine to humans and other animals but (p 144) the methods for identifying diiodotyrosine were generally open to criticism The fact that identical metabolites of thyroxine appeared in the urine when the thyroxine was labelled in the 3 5 or 3 5 positions casts doubt on the likelihood of diphenyl ether rupture occurring *in vivo* Fletcher[492] has stated that the diiodotyrosine which is found in the urine of patients treated with large doses of ^{131}I would most likely be formed as a hydrolytic product of circulating thyroglobulin released by thyroid irradiation Evidence for enzymic scission of diphenyl ether linkages appears in the work of Lissitzky and Bouchilloux[973], these authors found that mushroom tyrosinase split 3 3 -diiodothyronine to yield 3 monoiodotyrosine and other products Allegretti[29] has claimed that incubation of thyroxine with liver homogenate leads to the formation of 2 6 diiodo *p* quinone and 3 5 diiodophenylalanine these compounds were not however satisfactorily characterized and the work awaits confirmation

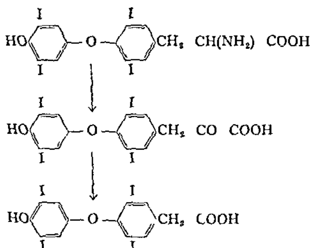
(V) Oxidative Deamination of the Iodothyronines

Oxidative deamination of the alanine side chain of the iodothyronines is not known to occur in normal animals although it has been demonstrated in various unphysiological situations Roche *et al*[1363] administered large doses of labelled thyroxine and triiodothyronine to thyroidectomized rats and later examined the bile on chromatographic analysis two new spots appeared These are referred to as B and II in Fig 20 (Chapter 7) They were tested with a variety of reagents and found to react with semi carbazide and *o* phenylenediamine which indicated that they possessed a carbonyl group from this it was concluded that the α keto acid analogues had been formed Myant[1137] has also detected the keto acid analogue of thyroxine in human bile

Some time ago Pitt Rivers[1223] suggested that the acetic acid analogues of thyroxine and triiodothyronine which both possessed physiological activity might be metabolites of the parent amino acids and could be derived from them by oxidative deamination followed by decarboxylation Later interest in these analogues was stimulated by the claim[1601, 1597, 1598, 1599, 1600] that they exert an immediate stimulation of oxygen consumption on rat kidney slices *in vitro* and of metabolic rate *in vivo* Since then an active search has been made for the thyroacetic acids in mammalian

tissues, triiodothyroacetic acid has been detected in extracts of rat bile[1080], kidney and muscle after injection of ^{131}I labelled triiodothyronine in rats [1352, 1351], small amounts have also been found in the plasma of guinea pigs after triiodothyronine administration[514]. Tetraiodothyroacetic acid is reported to be present in the plasma of hepatectomized dogs after injection of thyroxine[497].

The thyroacetic acids have also been found in the kidneys and liver of mice which had been injected with large doses of radioactive iodide (Galton and Pitt Rivers, unpublished). It appears therefore that these acetic acids are true metabolites of the endogenous hormones and are not only formed after administration of unphysiological doses of thyroxine and triiodothyronine. Although the thyropyruvic acids have not been detected under the more physiological experimental conditions, it is likely that one metabolic pathway for the thyroid hormones goes through the keto acids to the acetic acids.

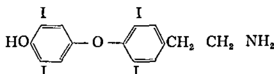


Oxidative deamination of thyroxine and triiodothyronine to the corresponding acetic acid analogue by slices and homogenates of brain tissue has also been described[1578, 1572]; further Tomita *et al* [16 7] have prepared a soluble enzyme from rat kidney which can catalyse this reaction. In all these experiments, however, chromatographic identification alone has been used. Tata and co-workers[1578] have pointed out that the thyroacetic acids are chromatographically indistinguishable from corresponding thyroid hormone analogues where the alanine side chain is replaced by a propionic acid group or a carboxyl group. Some caution should therefore be observed in making claims for the biological existence of these acetic acids. Tomita [1626] has however demonstrated the conversion of ^{14}C labelled 3:5 diiodothyropyruvic acid to 3:5 diiodothyroacetic acid, and it is likely on general

grounds that this reaction should occur rather than deamination to a propionic acid, which has no biological parallel From a quantitative point of view oxidative deamination of the thyroid hormones is less important as a metabolic pathway than deiodination and phenolic conjugation Whether these compounds are important in the thyroid hormone cycle is not yet clear, they may merely represent breakdown products of thyroxine and triiodothyronine which can later undergo conjugation and deiodination like the parent compounds[1353, 1719]

(VI) Decarboxylation

Thibault and co workers[1599] have investigated the activity of thyroxine and other compounds in potentiating the effect of adrenaline on intestinal muscle This potentiation by thyroxine is only observed after a latent period but an immediate effect is obtained with thyroxamine



This compound was synthesized many years ago by Ashley and Harington [57] it has very little thyroxine like activity in mammals Triiodothyronine also potentiates adrenaline activity in muscle but again only after a latent period whereas the effect of triiodothyronamine is immediate [02] Attempts have been made[895] to detect thyroxamine in muscle extracts after incubation with thyroxine but the identification was inconclusive It is unlikely that either of these amines has any physiological significance

CHAPTER 9

DISEASES OF THE THYROID

ONCE the cause of a disease is understood problems of diagnosis and treat ment usually resolve themselves The limited space available here for consideration of clinical matters has therefore been wholly devoted to a discussion of aetiology Combined pressure from biochemical physio logical and clinical studies has resolved many minor problems concerning the nature of thyroid diseases but has left outstanding two major ones the causation of sporadic goitre and the causation of Graves disease In this chapter evidence bearing on these two problems will be examined some of it derived by analogy from our much greater knowledge of the aetiology of rarer conditions In a separate small section some points relating to the aetiology of thyroid cancer are discussed briefly

(I) NON TOXIS GOITRE

This term is used to include all goitres not associated with hyper-thyroidism In those cases in which the aetiology is at least partially understood the thyroid enlargement can be regarded as compensating (or attempting to compensate) for some difficulty in the manufacture of thyroid hormone In the first condition to be discussed the difficulty is caused by lack of iodine and compensation is usually successful In the second and third it is caused by ingestion of an antithyroid substance or by a genetically determined enzyme defect in these conditions compensation is usually less successful and hypothyroidism results The analogies provided by these three types of defect can then be made use of in an attempt to under stand sporadic goitre that is non toxic goitre as it occurs in areas of relative iodine abundance such as London or Boston The assumption is made—without any direct evidence—that in these cases too the goitre is essentially a compensatory phenomenon and that what we have to seek is an under-lying defect in hormone synthesis

(a) *Endemic Goitre*

Kelly and Snedden[342] have shown in an extensive review of world literature that goitre occurs in every country of the world in which it has been looked for The difference between endemic and non endemic areas is therefore only relative It is however useful to retain these terms when

considering aetiology A loose but practical definition is to describe as endemic an area where the majority of the inhabitants have goitres

Older work on the relation of iodine intake to goitre incidence has been extensively reviewed by Orr and Leitch[1178] This work showed that in endemic goitre areas the iodine content of water soil and locally grown food crops was usually lower than elsewhere These observations can be accepted in general terms, but cannot be relied on in detail because of imperfections in the methods of iodine analysis then in use

Even a precisely inverse correlation between iodine intake and goitre incidence would not finally prove a causal connection The theory that endemic goitre is caused by iodine deficiency has, however, been strongly supported by three recent investigations (i) the production of goitre in experimental animals (ii) biochemical and isotopic studies in endemic goitre areas and (iii) the unequivocal demonstration that the administration of iodine prevents goitre

(i) *The production of goitre in experimental animals* has often been attempted but the results have not been satisfactory until recently because low iodine diets are grossly abnormal and it could be argued that they may contain positive goitrogenic factors Axelrad *et al*[81] have now shown that mice on an iodine deficient (Remington) diet develop thyroid enlargement even when each item of the diet is removed separately The only item in the diet which appeared to have any goitrogenic effect was surprisingly sodium chloride However thyroid enlargement, preventable by iodine supplements, could be demonstrated whether or not sodium chloride was present

(ii) The kinetics of iodine metabolism in the inhabitants of an endemic goitre area in the foothills of the Argentinian Andes has been studied by Stanbury *et al*[1519] These people had very high uptakes of isotopic iodine, inversely correlated with their daily output of stable iodine Their stores of organic iodine in the thyroid were low When they were given supplementary iodide the uptake of radioactive iodine slowly reverted towards the values usually found in non endemic areas All these findings are entirely compatible with the hypothesis that goitre in Mendoza is caused by lack of iodine in the diet Roche *et al*[1371] made similar studies in an endemic goitre area in Venezuela They found that ^{131}I uptake among the inhabitants of this region was equally high whether or not they had goitres This observation suggests that some thyroids can compensate for iodine deficiency by hyperfunction without an obligatory increase in size

(iii) Scrimshaw *et al*[1437] conducted a well controlled trial of the effect of potassium iodide and iodate on the incidence of goitre in school children from endemic areas in El Salvador and Guatemala A control group given a placebo was included and the observers did not know which children

had been treated There was a marked fall in goitre incidence in the treated groups Although similar results had been obtained by previous workers this was the first trial *in which observer bias was clearly eliminated*

Goitre can be produced in animals by iodine deficiency alone Iodine metabolism in endemic goitre areas is what would be anticipated if the goitres were caused by iodine deficiency Endemic goitre can be prevented by iodine supplements It seems safe to conclude that endemic goitre is caused by iodine deficiency

(*b*) *Drug Goitre*

Goitre or hypothyroidism or both have been recorded in patients treated *with thiocyanates (for hypertension)*[100 101 1273] *in patients with leg ulcers* to which resorcinol had been applied [44] with *p* aminosalicylic acid (P A S) in tubercular patients[873 284 996 67 214] with phenylbutazone (Butazolidine) in patients with rheumatic disorders[1108 913] with cobalt salts in patients (mainly children) with refractory anaemias[647 880 558 211 661] with large doses of iodides mainly in patients with asthma[139 1106 7 5 1650 1397 1160] and with iodinated antipyrine (iodopyrine) again in asthmatic subjects[1330 1016 1639] The antithyroid action of these various drugs differs Thiocyanates prevent the concentration of iodide without affecting *organic binding of iodine the goitres should therefore be prevent*able by iodide administration (as well as by thyroid extract) P A S on the other hand acts like the thiourea compounds[423] and the goitres are preventable by thyroid extract but not by iodide[996 14] Phenylbutazone also has a thiourea like action[1106] The anti thyroid effect of resorcinol is evanescent and it can probably only cause goitre if continuously absorbed [392] The anti thyroid action of cobalt has been questioned but it appears from the work of Kriss *et al* [681] that there are considerable species differences in the response to cobalt and also that the dose may be critical The antithyroid effect of large doses of iodide is poorly understood Iodide in high concentration can inhibit the organic binding of iodine by surviving thyroid slices[1115] and by the intact rat thyroid[1743] but the effect only lasts for 24–48 hr[1744] and organic binding is resumed in spite of continued iodide administration In man a similar type of effect has been found *in in vivo* studies with ^{131}I [1528 8] *doses of the order of 1 mg of* potassium iodide affect uptake in the same sort of way as thiourea com pounds It is not known whether this effect is transient or not Iodopyrine in doses of 10–60 mg acts similarly this may be a specific effect of the molecule or may result from a secondary release of iodide Goitres caused by iodide or iodopyrine are certainly rare in relation to the number of persons who regularly consume these substances therapeutically It may well be that all the thyroids which enlarged on this type of medication had an underlying lesion of some sort This seems to have been the case

in the patient reported by Hydovitz and Rose[785] in which hypothyroidism was only partially relieved when iodopyrine was discontinued

Certain general conclusions can be drawn from a study of cases of drug goitre First, an essential requirement for the production of this type of goitre is that the drug be taken regularly and for a period of at least several months It is unlikely that drugs are taken with as much consistency as their prescribers believe Dixon *et al* [380] have recently shown that more often than not P A S is absent from the urine of patients supposedly consuming large and regular doses This probably explains the relatively low incidence of goitre in P A S treated patients, and indeed the low incidence of drug goitre in general Drugs are probably only taken with complete regularity by the obsessional and by those in whom the omission of a dose has unpleasant effects as in some cases of rheumatism treated with phenylbutazone and in some asthmatics treated with iodide or iodopyrine

The second general conclusion about drug goitre is that it is more often than not accompanied by some degree of hypothyroidism, indeed some of the reported cases had myxoedema without an obvious goitre Astwood[68] has remarked that the thyroid is much more efficient at compensating for ✓ iodine deficiency than for the effect of an antithyroid substance

Thirdly it is worth noting that the sex ratio in those drug goitres in which there are sufficient numbers of described adult cases (P A S and iodide) is apparently that of the underlying disease for which the drugs had been prescribed Antithyroid substances apparently affect male and female thyroids to a roughly equal extent

(*c*) *Sporadic Goitrous Cretinism*

In the classical endemic goitre areas cretinism was relatively common, and was usually associated with goitre[1412] It would seem that in these cases iodine deficiency was a factor in the causation of cretinism since the frequency of this condition has greatly decreased in Switzerland since the introduction of iodine prophylaxis[1152] Clements[288] has however, pointed out that the geographical distribution of cretinism is by no means parallel to that of endemic goitre cretinism is rare or absent for instance in the Jura and in the Andes where a high intensity of goitre has been known to be present for several centuries Clements makes the interesting suggestion that cretinism only occurs on a large scale in those endemic areas where inbreeding has favoured a build up of genes which affect thyroid function adversely The classical endemic goitre areas were mostly narrow valleys among the high mountains having little contact with the outer world The observation that diiodotyrosine may be present in the blood of endemic cretins[313] favours this suggestion, iodine deficiency alone could not account for this finding

In non endemic areas cretins generally have no goitre In such cases

the thyroid has usually failed to develop Occasionally a tiny fragment of thyroid may be present in the base of the tongue[1059, 1630] probably it was unable to expand to an adequate size in that situation

In a review of cretins drawn from various parts of the North American continent, Osler[1150] noted that a minority had goitres Cases of this type have in recent times been very extensively investigated by isotopic and chromatographic techniques The subject has been extensively reviewed by Lelong *et al* [944], Stanbury and Querido[1526] and Stanbury and McGirr [1522] In a large proportion of the recorded cases there is a familial incidence of the condition and consanguinity is common among the parents From the genetic point of view the most informative group of cases is that studied by Hutchison and McGirr[779] in south west Scotland These cases all derived from a family of itinerant tinkers whose pedigree has been traced back to an Irish immigrant who married his Scottish cousin 160 years ago The family was isolated from the rest of the community and there was much intermarriage in the second and third generations all the affected children were born of normal but consanguineous parents There seems little doubt that in this family goitrous cretinism is caused by a recessive gene Such genetic data as are available about cases from other parts of the world are compatible with the same hypothesis

From the biochemical aspect Stanbury has differentiated three types of defect leading to goitrous cretinism (i) failure to form any organic iodine containing compounds (ii) failure of iodotyrosines to couple together to form triiodothyronine and thyroxine, and (iii) absence of the enzyme (deiodinase) which breaks down unused iodotyrosine radicals and so makes their iodine available for re utilization as iodide The first group is easily recognized for thiocyanate or perchlorate causes the discharge of all labelled iodine held within the thyroid[1520, 1517, 1525, 1431, 805] It seems probable that in these cases the enzyme responsible for the oxidation of iodide is missing Stanbury s explanation of the second type is plausible but as yet unproved one case described by Stanbury *et al* [1525] and one by Werner *et al* [1707] appear to belong to this group The hypothesis is that in such cases iodotyrosines are formed normally but that the enzyme which enables them to couple is missing This enzyme has never been positively identified, so that the hypothesis is not at the present time directly verifiable The observation that at thyroidectomy after a dose of ^{131}I most of the labelled iodine in the thyroid was present as mono and diiodotyrosine supports Stanbury s views

Stanbury s hypothesis that in the third group of cases goitrous cretinism results from lack of deiodinase is extremely ingenious but not yet supported by firm evidence The agreed facts are as follows in the normal thyroid iodotyrosine and iodothyronine radicals are formed within the thyroglobulin molecule Not all the iodotyrosines are able to couple to

form iodothyronines, so that when proteolysis occurs both these two types of compound are released All the free iodotyrosines are broken down by the deiodinase which is demonstrably present, iodide being released and re entering the synthetic cycle No iodotyrosines escape from the thyroid so that it is not surprising that no specific deiodinating enzymes have been demonstrated elsewhere in the body But if iodotyrosines are injected intravenously *in a normal subject they are rapidly* broken down and their iodine is excreted in the urine as iodide, or taken up by the thyroid for hormone synthesis Stanbury s hypothesis supposes that these arrangements have broken down to such an extent that an iodine deficiency arises which is so extreme as to cause goitrous cretinism *This hypothesis seems improbable because (i) it is doubtful* whether any degree of iodine deficiency short of absolute could cause cretinism (though it might well cause goitre alone) (ii) it involves a simultaneous breakdown of what are probably distinct mechanisms i e deiodination in the thyroid attributable to a specific enzyme, and deiodi*nation elsewhere due to a more general metabolic activity of tissues* Nevertheless Stanbury has brought forward a certain amount of evidence in favour of his hypothesis of which by far the most substantial part is the demonstration in a single case of goitrous cretinism, that the thyroid deiodinase was absent[1250] His conclusions concerning the apparent *inability of goitrous cretins to deiodinate labelled intravenous iodotyrosines* [1253] are based on chromatographic methods which are open to criticism (see p 148) further Mosier *et al* [1122] have shown that two goitrous cretins became able to deiodinate normally when treated with thyroid extract. Inability to deiodinate iodotyrosines peripherally may merely be a secon*dary effect of hypothyroidism*

At the present time it seems best to divide goitrous cretins into only two groups those who cannot make organic iodine compounds of any sort and those who can make iodotyrosines but have difficulty in converting them into iodothyronines In both groups there is satisfactory evidence that the defect is genetically determined and presumptive evidence that it manifests itself biochemically as an enzyme failure It is perhaps worth bearing in mind that the biochemical defect does not necessarily imply absence of an enzyme In Stanbury s first type, for instance a metabolic abnormality outside the thyroid might permit a build up of a substance having thiourea like activity The analogy of other inborn errors of metabolism suggests that it would be unwise to assume without direct evidence that the primary biochemical abnormality is necessarily located in the thyroid

(d) Goitre with Congenital Deafness

Deaf mutism is traditionally an accompaniment of endemic goitre Clements[288] has pointed out that it is not necessarily a result of iodine

deficiency the isolated populations of the mountain valleys where endemic goitre classically occurs would be subject to periodic epidemics of rubella among the adults Maternal rubella during pregnancy is now a well-recognized cause of congenital deafness The same situation would also predispose the population to genetic defects as a result of inbreeding including inherited forms of deafness

The relationship of deafness to goitre is hard to disentangle in an endemic goitre area where by definition, almost everyone has a goitre That there can be some sort of genetic connection between the two conditions emerges clearly from the cases of sporadic goitre with congenital deafness described by Brain[210] Deraemaeker[368] Thieme[1602] and Morgans and Trotter[1110] In all the published cases two or more siblings were affected but not their parents All cases had both deafness and goitre, neither defect occurring singly All the goitrous subjects were euthyroid and had developed normally There is therefore no question of the deafness being a result of thyroid deficiency Both defects seem to be the result of a single defective gene which appears to be recessive in the families so far described It is however not obvious how a single gene defect could affect structures so embryologically remote as the acoustic nerve and the thyroid gland Perhaps the only connection is biochemical an enzyme block elsewhere might enable metabolites to build up which could affect both structures In Morgans and Trotter s[1110] two cases the administration of perchlorate partially discharged iodine from the thyroid, they therefore seemed to have a minor degree of the same sort of defect as Stanbury s first type of goitrous cretin It is conceivable that as the result of an enzyme block in another tissue a metabolite with a thiourea like action had been released into the circulation this would affect the thyroid in the manner observed by Morgans and Trotter[1110] The same enzyme block might have a comparable effect on the acoustic nerve perhaps acting through a quite different metabolite Some such theory seems to be required by the genetic evidence which points to a single gene defect as the cause of two embryologically unrelated lesions

(*e*) *Sporadic Goitre*

Sporadic goitre is eight times more common in women than in men[1280] *In this it differs from endemic goitre drug goitre goitrous cretinism and* goitre with congenital deafness in all of which conditions the sex ratio is approximately one to one Endemic goitre hardly provides a fair comparison since if practically all the population is affected the ratio can scarcely fail to approach unity The other conditions provide a reasonable analogy from which to argue Since antithyroid substances and genetic defects affect men and women equally neither can be the whole explanation of sporadic goitre

Sporadic goitre in contrast to drug goitre and goitrous cretinism is never associated with hypothyroidism This at once suggests a different underlying mechanism and a closer resemblance to endemic goitre In the cases of endemic goitre studied by Stanbury *et al*[1519] serum protein bound iodine was normal The thyroid has an almost unlimited capacity to compensate for iodine lack but is less successful at overcoming enzyme defects There would therefore seem to be a *prima facie* case for supposing that sporadic goitre is also the result of iodine deficiency, particularly as it is never possible to draw more than an arbitrary line between sporadic and endemic goitre This hypothesis is more directly supported by the observations of Burrell and Fraser[248] Five to twenty per cent of cases of simple goitre in areas like London have an abnormally high uptake of radioactive iodine but uptake is reduced to normal by a short course of treatment with small doses of potassium iodide

The main difficulty encountered in applying the iodine deficiency hypothesis to sporadic goitre is the need to explain why only a relatively few people, presumably consuming similar diets develop goitre in non endemic areas The most obvious explanation would be that their diets are not in fact similar in respect of iodine This is an explanation which could be but has not been tested in the field for though many comparisons of the iodine uptakes of inhabitants of different regions have been made no one seems to have compared the iodine consumption of goitrous and non-goitrous subjects all living in the same area A hint that there may be differences comes from the observation by Cochrane Miall and Trotter (unpublished) that people with goitres eat significantly less fish than randomly selected non goitrous subjects living in the same area Nevertheless differences in dietetic habits can scarcely be the whole explanation, for they do not account for the remarkable sex difference It seems likely that persons with sporadic goitre are in addition losing iodine by some route not yet defined, possibly by the kidney for Cassano *et al*[262] have reported abnormally high renal clearance rates for iodide in eight subjects with sporadic goitre This observation does not of itself explain the greater susceptibility of women, and it would seem worth while to enquire whether there is any excessive menstrual loss of iodine in females with goitre

However iodine deficiency may be produced it is probably the major cause of sporadic goitre There may well also be ancillary causes Genetic factors are particularly hard to disentangle when there is a strong suspicion that familial dietetic habits are themselves involved in the aetiological situation Eugster[459] in his massive study of twins with goitre found that the main influences at work were environmental This was in Switzerland genetic factors might be easier to study in a non endemic area An odd observation is that persons with sporadic goitre are less often able to taste phenylthiourea than normal persons[710], the ability to taste this and other

antithyroid substances is *inherited in a well defined manner*[709] *It would* be of considerable interest to know whether differences in the renal excretion of iodide are inherited, as defects of tubular reabsorption often are

Though the ingestion of goitrogenic substances in the diet is unlikely to be of major importance it probably contributes to the production of goitre in special circumstances McCarrison s[1060] experiment seemed to show *that polluted water can cause goitre* though the odd feature was that the goitres appeared in as short time as 10 days Hettche[735] has isolated a goitrogen from polluted water and identified it as urochrome Steyn *et al*[1543] think that an excessive intake of fluoride may cause goitre in some parts of South Africa. Taylor[1594] has suggested that too much *calcium may be a contributory cause of goitre* Astwood *et al*[72] isolated 5 vinyl 2 thiooxazolidone (a thiourea derivative) from swedes Astwood[68] has discussed the possibility that this substance may be a cause of goitre In swede and turnip roots it is present in inactive combination, from which it can be relased enzymically on soaking in water Cooking destroys the enzyme, so that these roots are only goitrogenic when eaten raw This substance has not been found in cabbage leaves but Suk[1552] presented epidemiological evidence that an excessive intake of cabbage was responsible for the high goitre incidence in certain Carpathian villages Clements and Wishart[289] tried to explain an increased incidence of goitre in children in parts of Tasmania in spite of iodine prophylaxis The increased goitre incidence coincided with the introduction of a free milk scheme for school children To meet the extra demand for milk farmers had *taken to feeding* their cows on chou moellier (a type of kale) Clements and Wishart showed that milk from these cows inhibited ^{131}I uptake in man

Most of these instances of goitre supposedly due to the ingestion of antithyroid substances occurred in special circumstances and *do not seem relevant to present day conditions in this country* The possibility that milk from cows fed on brassica crops (or with rape seed concentrates) may be goitrogenic might however be relevant and should be investigated

So far attention has been directed to the possible primary causes of *sporadic goitre It is also worth considering whether there* is not in addition some mechanism at work which makes a goitre—whatever its original cause—self perpetuating Clinical evidence favours this hypo thesis Taylor[1595] has shown that goitres start as a simple diffuse hyperplasia of the thyroid but over the course of years alternating cycles of *degeneration and regrowth affect small areas of the gland independently* The result is a very heterogeneous structure—the nodular goitre In general Taylor points out goitres in their diffuse stage can be suppressed by the administration of thyroxine but nodular goitres cannot This

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picture needle biopsies are not an adequate substitute for full scale sections Lymphadenoid goitre is often called "Hashimoto s disease or Hashimoto s thyroiditis" These terms seem to the writer to be acceptable alternatives in spite of the tendency of certain purists to insist on strict conformity with Hashimoto's original description Struma lymphomatosa ' is another synonym in current use

Skillern *et al*[1479] and Goudie *et al*[603] consider that spontaneous myxoedema and lymphadenoid goitre are aetiologically related The points in favour of this hypothesis are (i) both occur predominantly in middle aged females, (ii) hypothyroidism is by definition a feature of spontaneous myxoedema and occurs sooner or later in nearly all cases of lymphadenoid goitre (iii) the histological picture is very similar in the two diseases, though in some cases of myxoedema the process has reached the stage of complete fibrous replacement of all thyroid tissue in less advanced cases the histological picture may be indistinguishable from that of lymphadenoid goitre (iv) in both diseases ^{131}I uptake cannot be increased by thyroid stimulating hormone (TSH) (v) high erythrocyte sedimentation rates and abnormal serum flocculation tests have been found in a high proportion of patients with both diseases (vi) antibodies to thyroid extracts while commoner in lymphadenoid goitre also occur in spontaneous myxoedema A further argument which seems to point in the same direction is that cases are not infrequently encountered in which it is difficult to say whether spontaneous myxoedema or lymphadenoid goitre would be the more appropriate diagnosis In these cases there is a greater or less degree of hypothyroidism associated with a just palpable very firm thyroid The slight degree of thyroid enlargement scarcely justifies the name of goitre yet is not compatible with the usual idea of the atrophied thyroid of spontaneous myxoedema

On this view the only material difference between spontaneous myxoedema and lymphadenoid goitre would result from differing responses to oversecretion of TSH by the pituitary (which will inevitably follow a fall in the level of circulating thyroid hormone) Spontaneous myxoedema indicates a failure of the thyroid to respond to TSH by enlargement whereas lymphadenoid goitre could be regarded as partially compensated hypothyroidism It is interesting to note that compensation is commonly only partially successful as compared with the adjustment to environmental iodine deficiency by thyroid enlargement

When obvious clinical hypothyroidism is present the uptake of ^{131}I by the thyroid is low and may even be undetectable Very low uptakes are commoner when the thyroid is not enlarged but may occur even in the presence of a goitre Normal uptakes are a common finding in lymphadenoid goitre but may also occur with mild degrees of hypothyroidism even in the absence of a goitre in such cases there is no response to 10 units of

difference in behaviour of the two stages of goitre may be reflected in the altered biochemical behaviour of nodular goitres Pitt Rivers *et al*[1228] showed that these goitres contain less thyroxine relative to iodotyrosines and less diiodotyrosine relative to monoiodotyrosine Trunnell and Wade[1644] made similar observations in two nodular goitres and suggested that they indicated a reversion towards a more primitive type of synthesis They also showed that during embryological development of the chick the thyroid is first only able to concentrate iodide then becomes able to make monoiodotyrosine, then diiodotyrosine and finally thyroxine It seems reasonable to suggest that a long period of continual production of new thyroid tissue might eventually result in a cell population part of which was immature biochemically

It seems to the present writer that the most plausible hypothesis concerning the aetiology of sporadic goitre would be summarized as follows the major initiating factor is iodine deficiency, determined partly by food habits and partly by differences in iodine utilization, minor contributory causes may be the ingestion of goitrogens and genetic factors whose mode of action cannot be specified the process of goitre formation, once initiated has an inherent tendency to be self-perpetuating owing to the inability of a tissue to reproduce itself indefinitely at its highest level of functional activity

(II) Spontaneous Myxoedema and Lymphadenoid Goitre

Spontaneous myxoedema is used to mean acquired hypothyroidism without goitre The condition is often known as 'primary myxoedema' to distinguish it from hypothyroidism secondary to pituitary failure

Lymphadenoid goitre is applied to a type of goitre (usually diffuse) in which histological examination shows infiltration by lymphocytes and plasma cells a variable increase in fibrous tissue destruction of acinar epithelium with an increase in cell height and the presence of large oxyphilic (Askanazy) cells Some authors have laid down various strict criteria for the diagnosis of this condition, but these pronouncements have little value since they were made in ignorance of the aetiology of the condition Recent work suggests that an immunological process may be the cause of this type of goitre and therefore the infiltration with lymphocytes and plasma cells would seem to be the most significant histological feature At the present time when rapid progress is being made in our understanding of this condition it would seem inadvisable to insist too rigidly on the presence of this or that histological feature before the diagnosis is permitted It may well be that the ultimate diagnostic criterion will be serological rather than histological In any case with the present tendency to treat patients with this disease medically histological material is now seldom available Owing to the great variability in the histological

untreated cases the antibody level in the serum is very high sometimes as much as 5 mg of antibody protein (against thyroglobulin) per ml of serum[387] and is reflected in a high level of circulating gamma globulin, with the usual accompaniments of a high erythrocyte sedimentation rate and abnormal flocculation reactions It seems likely that these extremely high antibody levels are responsible for part at least of the pathological changes in the thyroid Since one of the two antigens is believed to be intracellular it seems reasonable to expect that the corresponding antibody would be the more damaging This would lead to rupture of the acini with release of thyroglobulin into the circulation and antibody formation against this component Formation of antibodies against thyroglobulin is immunologically plausible, since this substance would not be in direct contact with blood during foetal life and tolerance to it would therefore not develop The idea that the thyroid changes in lymphadenoid goitre result from an auto immune reaction is supported by Rose and Witebsky s[1379] demonstration that rabbits can form antibodies against rabbit thyroid extracts and when immunized in this way tend to develop destructive thyroid lesions with some cellular infiltration The thyroid of a dog treated in this way in Witebsky s laboratory showed a histological picture strongly resembling that seen in human lymphadenoid goitre In the rabbit and dog experiments Freund adjuvants were used to secure high antibody levels Since these materials can themselves cause immuno logical reactions the experiments of Rose and Witebsky[1379] cannot be taken as showing that an increase in circulating antithyroid antibodies is alone sufficient to cause the thyroid lesions Some other mechanism may also be required Similarly in the human cases the simple view that non specific damage to the thyroid releases antigen with the result that anti bodies are formed and react back on the thyroid is likely to require modification Probably some as yet unspecified factor must be present to enable antibodies to be formed in sufficient quantity or for the thyroid to react to them in a special way A case of Addison s disease with lymphadenoid goitre described by Morgans[1103] seems to point in the second direction when treated with physiological doses of cortisone this patient s large goitre almost disappeared although the level of circulating antibodies remained high Lymphocytic infiltration of the thyroid is commonly found in Addison s disease[1483]

Although the precise mechanisms at work are still uncertain it is fair to conclude that antibody formation is an essential factor in the development of lymphadenoid goitre Antibodies are also formed in a proportion of cases of spontaneous myxoedema[387, 603, 1641] Although the titres are usually lower than in cases of lymphadenoid goitre it seems reasonable to suppose that a similar process is at work here also In some of the cases the active stage of the disease may have occurred in the distant past so it

TSH intramuscularly, a dose which induces a brisk increase in ^{131}I uptake in normal subjects. Cases of lymphadenoid goitre are similarly unresponsive to TSH, whatever the initial uptake[1479, 386]. In lymphadenoid goitre ^{131}I uptake may be above normal limits. The rate at which thyroid radioactivity subsequently declines is also abnormally rapid in lymphadenoid goitre[386]. This finding and the lack of response to exogenous TSH can be taken as indications that the thyroid is already exposed to strong TSH stimulation from the patient's own pituitary.

In one instance a lymphadenoid goitre was examined chromatographically by Pitt-Rivers (reported in [386]) and showed an abnormally low proportion of thyroxine iodine. This suggests a biochemical deterioration in function similar to that found in nodular goitre. The process may have progressed even further in the case of lymphadenoid goitre, for Morgans and Trotter[1109] found that perchlorate given 1 hr after ^{131}I, usually caused a brisk discharge of radioactivity from the thyroid. This was taken to indicate a partial thiourea-like block similar to that found in Stanbury's first type of goitrous cretin, and in the goitre with congenital deafness syndrome. It is not possible to say whether this iodination defect is caused by the destructive (perhaps immunologically induced) process in the thyroid or whether it represents a biochemical inadequacy (possibly genetically determined) which preceded the histological changes usually regarded as typical of lymphadenoid goitre.

The most important advance in our understanding of these conditions came with the demonstration by Roitt *et al.*[1374] that the serum of patients with lymphadenoid goitre contains antibodies to antigens present in thyroid extract. These antibodies can be demonstrated by precipitin, tanned red cell or complement-fixation techniques. The antibodies are specifically directed against thyroid tissue since they do not react with extracts of other organs and are true auto-antibodies since a patient's serum may react with his own thyroid[1711, 1736]. Two distinct antigen-antibody systems may be involved in lymphadenoid goitre, one giving strong precipitin reactions but weak complement fixation in which the antigen is almost certainly thyroglobulin, the other giving strong complement fixation but weak precipitin reactions which is as yet unidentified[1641]. Both antigens can be found in any human thyroid gland, but the second (unidentified) antigen may be present in greater quantity in toxic diffuse goitres. White's[1711] studies with fluorescein-labelled antibody showed the main body of antigen to be in the colloid, but there was also some in the acinar cells. Our observations have shown that whereas thyroglobulin can readily be removed by soaking thyroid slices in saline, the other antigen remains behind, suggesting that it is located in the cells. Virtually all cases of lymphadenoid goitre have one or both of the antibodies in their serum, though the level declines markedly after a radical thyroidectomy. In some

description which was based on only three patients and could not possibly include all the manifestations of this very variable condition

Another problem of definition immediately presents itself namely whether hyperthyroidism occurring in a patient with a pre existing nodular goitre (secondary thyrotoxicosis toxic nodular goitre Plummer s disease) should be included under the general heading of Graves disease This question has been debated for a long time and still remains open The evidence both for and against the identity of the two types of hyper thyroidism seems basically unsatisfactory and not worth presenting in detail An arbitrary decision has therefore been taken to exclude from this section those cases ordinarily referred to as secondary thyrotoxicosis or toxic nodular goitre Even after disposing of this question in a somewhat high handed fashion the problem of assembling in any coherent form the few scraps of useful information we possess about the causes of Graves disease is sufficiently formidable and will be dealt with piecemeal under separate headings

Epidemiology

When aetiology is wholly unknown epidemiological studies often give the first useful clue They have failed to do so in the present instance partly because they have been seldom attempted partly because of the formidable diagnostic problems involved As soon as one attempts the apparently simple task of ascertaining the incidence of this condition in a defined population it becomes apparent that a large proportion of possible examples have had thyroidectomies performed for what may or may not have been Graves disease in different hospitals and at varying times and that no amount of research will enable a firm retrospective diagnosis to be made

The most comprehensive attempt to plot the distribution of Graves disease was made by Sallstrom (quoted in [803]) who reviewed hospital records from the whole of Sweden His conclusions were unfortunately meagre There was a tendency for the condition to be more frequent in urban than in rural areas but there was also a correlation between the incidence in any given town and that in the surrounding countryside The incidence of Graves disease was not correlated with that of simple goitre but appeared to be more frequent in areas with a continental as opposed to a maritime climate

The only other major epidemiological contribution is also Scandinavian Iversen s[803] very thorough study of the remarkable epidemic of Graves disease which occurred in Denmark at the end of the Second World War His survey was based like Sallstrom s, on hospital statistics covering the whole country with more detailed studies in Copenhagen itself There seems no doubt that the incidence of Graves disease increased by a factor

is not surprising that antibodies are not found as consistently as in lymphadenoid goitre

When hypothyroidism is encountered in the clinic it is almost always possible to discover a definite thyroid lesion caused either by disease (spontaneous myxoedema lymphadenoid goitre hypopituitarism) or by therapy (antithyroid drugs thyroidectomy or irradiation) In mild cases ^{131}I uptake may fall within the normal range, but does not respond to exogenous TSH[1480] It is however theoretically possible that the same *symptoms could be caused, not by failure of the thyroid to make its hor*mones but by failure of the peripheral tissues to respond to them No gross case of myxoedema has yet been reported without either a thyroid or a pituitary lesion Kurland *et al* [892] have however recently described a syndrome in which the response to thyroid hormones is alleged to be inadequate These cases have low BMR s but normal ^{131}I uptakes and normal PBI s they are said to have non myxoedematous hypometabolism and are alleged to respond to triiodothyronine but not to thyroxine In a further attempt to define this syndrome Kurland *et al* [891] have studied the metabolism of injected labelled thyroxine The turn over rate of the labelled material was slightly greater than in normal subjects but the total quantity of thyroxine degraded per day appeared to be virtually identical in the patients and the controls The differences observed do not seem to be sufficiently pronounced to define the syndrome of non myxoedematous hypometabolism in a useful way It might have been more helpful if the response of these cases to triiodothyronine (and their failure to respond to thyroxine) had been more securely established by a double blind trial From both practical and theoretical points of view it seems highly desirable to establish beyond any doubt whether there are people who derive benefit from triiodothyronine but not from thyroxine This has not yet been done

(III) Graves Disease

In discussing hyperthyroidism and allied conditions considerable difficulties of nomenclature are again encountered This is an indication of our *fundamental ignorance of the aetiology of these states* It seemed best to use here the term Graves disease in order to include those cases with typical eye signs but normal thyroid function which cannot properly be included under terms such as hyperthyroidism thyrotoxicosis or toxic goitre Exophthalmic goitre is not a useful description since we need on occasion to include cases having neither a goitre nor exophthalmos There are objections also to the use of Graves disease since the condition was originally described not by Graves but by Parry but on the whole this seems the most useful term provided it is understood that all cases under this heading do not necessarily have to conform to Graves original

was to strengthen the case for regarding Graves disease and toxic nodular goitre as aetiologically distinct conditions

These and other studies have shown beyond doubt that some type of genetic abnormality is one of the factors causing Graves disease Presumably what is inherited is some type of predisposition to the disease, which only becomes manifest as the result of the operation of some undefined environmental stimulus Little progress is likely to be made in genetic studies of this condition until we have some way of recognizing this state of predisposition What should be sought for is some demonstrable abnormality in patients with Graves disease which is not itself a direct consequence of hyperthyroidism Exophthalmos and localized pretibial myxoedema might provide possible clues, since they can sometimes appear before the thyroid becomes overactive and do not respond to thyroidectomy or antithyroid drugs The common occurrence of vitiligo in patients with Graves disease might provide another clue this condition also does not seem to be affected by the actual state of thyroid function The studies of Ingbar *et al* [794] are potentially of great importance They showed that euthyroid relatives of patients with Graves disease have an increased uptake of ^{131}I and an increased rate of breakdown of labelled thyroxine when compared with controls This finding suggests that an augmentation of the rate of metabolism of thyroxine may precede any clinical signs of hyperthyroidism and might be the primary disturbance of function in Graves disease If so this would be the type of abnormality to study when genetic factors are being investigated

Clinical Course

Graves disease has been under close clinical observation for well over a century without any notable contributions to our knowledge of its aetiology However a small number of facts about the disease have been collected which will have to be taken into account in any final explanation of this disorder One is the preponderance of women in Graves disease (as in sporadic simple goitre spontaneous myxoedema and lymphadenoid goitre) the reported male/female ratios vary between 1 4 and 1 10[803] Another is the clinical course of the disease When patients with Graves disease are treated with antithyroid drugs hormone production by the thyroid is held down to normal levels but the underlying stimulus to the gland is not modified By reducing and then stopping the antithyroid drug it soon becomes obvious whether this stimulus is still active or not Observations of this type[1637] have shown that Graves disease is commonly episodic Active phases lasting a year or two often alternate with remissions lasting several years In some instances the interval between active phases may exceed the period of observation so it is possible that some patients have a single brief attack only remaining euthyroid for the rest

of between three and four during the years 1942–44, falling steeply in 1945. There would appear to be an obvious connection here with the German occupation of Denmark, but Iversen was not able to pin down the nature of the mechanism at work. The experience of Denmark contrasts with that of Belgium, where the incidence of Graves' disease declined during the same period. Iversen suggested that these events were in some way related to the very large changes in national diets resulting from the occupation. That in Belgium approached starvation level, whereas in Denmark the total caloric intake was not greatly lowered. There were, however, big qualitative changes and Iversen thought that one possibility was a decline in antithyroid substances normally present in the diet; he cited the complete cessation of imports of soya bean products as a possible example. Since it has never been shown that antithyroid substances occur in significant quantities in any ordinary diet Iversen's suggestion remains an untested hypothesis.

Genetics

The major contribution to our knowledge of the hereditary basis of Graves' disease also comes from Denmark, where Bartels[125] made a comprehensive study of the relations of patients with either Graves' disease or toxic nodular goitre. In the former condition he found evidence of a familial disposition in 60% of the cases. There was a significant increase in the incidence of Graves' disease compared with a control population not only among the sisters and mothers but also among the aunts of the patients with Graves' disease. Bartels thought the last finding was good evidence that the increased familial incidence resulted from genetic rather than environmental factors. A surprising feature was that relatives of patients with Graves' disease had an increased incidence not only of Graves' disease but also of simple goitre and myxoedema. He concluded that the data were compatible with the hypothesis that one or more recessive genes were able to cause an underlying instability of the thyroid which could express itself in different ways, and which had a penetrance of 70–80% in women, but much less in men.

A similar study in England by Martin and Fisher[1037, 1038] again led to the suggestion that a recessive gene was a factor in the aetiology of Graves' disease. Although this study also disclosed an apparent excess of cases of simple goitre among the relatives of patients with Graves' disease the authors thought that the more significant feature was that an excess of cases of Graves' disease was found among the relatives of patients with Graves' disease, but not among the relatives of patients with toxic nodular goitre. Their conclusion was that the influence of hereditary factors was clearly demonstrable in Graves' disease but remained in doubt so far as nodular goitre (toxic or non-toxic) was concerned. One result of this study

function of guinea pigs thyroids as compared either with pituitary extracts or serum from hypothyroid patients One possibility which has to be considered is that serum from cases of Graves disease contains a thyroid stimulating factor which is not derived from the pituitary

Patients with Graves disease who are overtreated with antithyroid drugs to the point of inducing hypothyroidism react with an increase in the size of their goitres This is a perfectly physiological response on the part of the pituitary and indicates that the output of TSH can be increased in these cases Therefore the output of TSH cannot have been maximal before hypothyroidism was induced Nevertheless it may have been sufficiently high to stimulate the thyroid

When patients have recovered from the hyperthyroidism of Graves disease either as a result of partial thyroidectomy or following a course of treatment with antithyroid drugs their ^{131}I uptake responds normally to thyroxine[1104] or to thyroid extract[1 09] The relatives of patients with Graves disease even though they have a high initial uptake also respond normally [93] These observations do not indicate any constitutional inability of the pituitary to respond to thyroid hormone which might precede or outlast the active phase of the disease

Present information about the state of pituitary function is ambiguous and leads to no clear conclusion It seems to the present writer that existing evidence is slightly but by no means decisively in favour of the view that the pituitary is functioning normally in this condition thyroid stimulation being caused by some other agent The fact that we only know of one substance which has this effect does not exclude the possibility that others exist Another possibility is that the thyroid becomes in some way sensitized to TSH

Exophthalmos

Exophthalmos is the best recognized of a series of bizarre changes in the eyes and surrounding tissues which often but by no means invariably accompany Graves disease The essential lesion appears to be an increase in the retro orbital contents As well as actually pushing the eyes forward this often leads to swelling of the subcutaneous tissues above and below the eyes and to conjunctival oedema (chemosis) and is often accompanied by a greater or less degree of ophthalmoplegia All these changes commonly occur together and appear to increase or decrease independently of the state of thyroid function

The situation is further complicated by the occurrence of yet another eye sign namely lid retraction or spasm of the upper eyelid This phenomenon causes a widening of the palpebral fissure giving an appearance often erroneously recorded as exophthalmos Lid retraction occurs in two contexts as an accompaniment of ophthalmoplegia (an attempted

of their lives In a minority the hyperthyroid phase appears to be unremitting and may last at a more or less constant level for many years perhaps for the duration of the patient s life

In the episodic type there is a tendency for relapses to be more frequent in the spring than at other times of the year[1105]

Pituitary Function

The only substance known to cause enlargement and overactivity of the thyroid is the thyroid stimulating hormone (TSH) produced by the anterior pituitary It has therefore often been postulated that excessive production of TSH might be the immediate cause of Graves disease This hypothesis has been made more attractive by Harris and Woods [07] demonstration that electrical stimulation of the hypothalmus of cortisone treated adrenalectomized rabbits increases thyroid activity This study has shown that there is a possible pathway by which events within the brain might influence thyroid function but the conditions of the experiment were somewhat artificial, in that the effects of simultaneous release of adrenocorticotrophic hormone (ACTH) had to be nullified by adrenalectomy and it is uncertain how far the conclusions can be applied to man

Considerations of this type have led to many attempts to measure TSH in the blood and urine of patients with different types of thyroid disease The methods and results have been reviewed by Adams and Purves[10] No technique at present available is capable of detecting TSH consistently in the serum of normal individuals although on physiological grounds it must be assumed always to be present On the other hand TSH is as a rule easily demonstrable both in blood and urine of patients with primary hypothyroidism In Graves disease results have been inconsistent but TSH has certainly been demonstrated in the blood of some cases[3 0, 346, 581] Adams and Purves[10] point out that these results may be significant, even if the amount of circulating TSH cannot be shown to be greater than in normals since the high levels of circulating thyroid hormone in these cases ought to have inhibited the production of TSH altogether Studies with radioactive iodine point in the same direction the mere fact that high uptakes of ^{131}I *can coexist with high levels of circulating thyroid hormone* can be taken as evidence of disturbed pituitary-thyroid relations in Graves disease Moreover the administration of additional amounts of hormone either as thyroid extract or as triiodothyronine does not decrease the high ^{131}I uptake of Graves disease[1, 09, 1708] These findings are compatible with two alternative hypotheses either the pituitary has ceased to respond to thyroid hormone or the thyroid is being stimulated by some other agent which operates without regard for the level of circulating thyroid hormone Adams and Purves[10] found some interesting differences in the time relations of the effect of serum from exophthalmic patients on the

minnow differed somewhat from those seen when anterior pituitary extracts were administered

The nature of the increase in orbital contents in exophthalmos also remains in some doubt. Rundle and Pochin[1401] showed that some 70% of the increase in orbital contents was attributable to fat a rather remarkable observation in a disease associated with wasting Many observations during the course of decompression operations on the orbit have shown that in the severest forms of exophthalmos (so-called malignant exophthalmos) the pathological picture is complicated by gross oedema of the orbital contents presumably a secondary effect of interference with venous drainage Another line of investigation by Asboe Hansen *et al* [55] showed that the skeletal muscles of patients with progressive exophthalmos contained considerable amounts of a hyaluronidase sensitive acid mucopolysaccharide On the assumption that a similar material is deposited in the ocular muscles the authors suggested that this finding might account for ophthalmoplegia and perhaps also for the increase in orbital contents Laurent and Scopes[914] reported that local injections of hyaluronidase caused marked improvement in a case of progressive exophthalmos

Localized Pretibial Myxoedema

The deposition of a mucinous material in the deeper layers of the skin of the lower part of the legs is another curious phenomenon associated with Graves disease In its clinical course pretibial myxoedema resembles exophthalmos in that it varies more or less independently of the state of thyroid function yet with a tendency to increase if the state of hyperthyroidism is abruptly controlled[164] Like exophthalmos (with which it is commonly associated) it has a relatively high incidence in males with Graves disease Histologically the deposition of mucin in areas of pretibial myxoedema is similar to but much more intense than the changes seen in the skin in generalized myxoedema Watson and Pearce[1698] showed that the skin in pretibial myxoedema contains a marked excess of acid mucopolysaccharides As might be anticipated from this finding the injection of hyaluronidase into the lesions causes a temporary decrease in size [609, 1088] At the present time the significance of this observation is obscure but it provides along with evidence previously quoted a hint that more detailed studies of mucopolysaccharides in thyroid disease might be rewarding

Emotional Changes

Patients with Graves disease are emotionally unstable They are readily provoked to tears or to outbursts of temper and they worry over trivialities All their reactions are rapid and highly charged with emotion These psychological changes may be the cause of the disease or its effect or a combination of the two The mere clinical observation of patients has not

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compensation for loss of upward movement of the eyes) and by itself when it seems to be more directly related to thyroid function, in that it usually disappears after partial thyroidectomy[4, 0].

The effect of controlling hyperthyroidism (either by antithyroid drugs, partial thyroidectomy or radioactive iodine) on exophthalmos and its associated signs is in any individual case, largely unpredictable. While in some cases an actual improvement has been recorded, the more frequent sequel to treatment directed against the thyroid is progression of the eye condition. For this reason physicians experienced in the treatment of Graves' disease tend to use antithyroid measures with some caution in the presence of any of the signs associated with exophthalmos. These signs may appear for the first time before there is any indication of thyroid activity, or not until treatment has brought about a condition of euthyroidism or hypothyroidism. Cases with gross exophthalmos are almost always treated with thyroid extract, but current opinion[1400, 381] is that this treatment has little effect on the eye condition except perhaps in cases with hypothyroidism. Irrespective of treatment, the general tendency is for exophthalmos to progress for a period of something like 6 months, afterwards becoming stationary or very gradually receding.

The tendency, indefinite though it is, for exophthalmos to vary inversely with the state of thyroid function has naturally suggested the idea that the agent causing the eye condition might be TSH, or an associated substance secreted by the anterior pituitary. A great many experiments have shown that something resembling human exophthalmos can be produced in a variety of experimental animals by the injection of pituitary extracts. The resemblance may be no more than superficial, since the bony orbit of man differs from that of other animals in being complete, hence the mechanical factors causing the proptosis may be quite different. Dobyns and Steelman[382] consider that the factor in anterior pituitary extracts which causes exophthalmos in the Atlantic minnow (*Fundulus*) is quite distinct from TSH. Experimental observations on exophthalmos are as confusing as the clinical data, and neither points to any clear conclusion.

Observations on TSH activity in the serum of patients with progressive exophthalmos likewise cannot be described as more than inconclusive. Gilliland and Strudwick[581], for instance, found that TSH was more frequently present and in larger amounts in patients with Graves' disease when exophthalmos was also present. The correlation with the clinical state was however not as close as would be expected if TSH were the principal agent causing exophthalmos. The absence of exophthalmos in patients with spontaneous myxoedema is presumptive evidence that TSH cannot be the sole operative factor. Dobyns and Wilson[383] have found an exophthalmos-producing substance in the serum of patients with progressive eye signs, but the time relations of the reaction of the Atlantic

These remarks are not intended to disparage attempts to find a psycho somatic explanation of the aetiology of Graves' disease This remains a perfectly possible explanation, particularly since Harris and Woods [07] demonstration of a physiological pathway connecting the hypothalamus to the thyroid by way of the pituitary All that it is intended to point out is that satisfactory evidence in favour of the psychosomatic hypothesis is more difficult to obtain than might appear at first sight and that the continued collection of evidence at an anecdotal level is not likely to be rewarding

Immunological Findings

It is well known to pathologists that the thyroid of patients with Graves disease often shows some degree of infiltration with lymphocytes Occa sionally the process is extensive and large areas of the gland have an appearance indistinguishable from that of lymphadenoid goitre[421] Levitt[961] pointed out that when large numbers of pathological thyroids are studied they can be arranged in a continuous series with purely hyperplastic changes at one end and the full picture of lymphadenoid goitre at the other His deduction that individual thyroids gradually evolve from the hyperplastic to the lymphadenoid stage is probably unjustified but it is difficult to avoid the inference that Graves disease and lymphadenoid goitre share some as yet undefined pathological process

As well as these histological similarities patients with Graves disease often have antibodies to thyroid extract in their serum which appear to be the same as those found in the serum of patients with lymphadenoid goitre though the titre is usually much lower These antibodies were detected by complement fixation in four out of ten patients with Graves disease by Trotter *et al* [1641] and in 62% of a much larger series by Goudie *et al* [603] The significance of this finding is at present obscure It may mean that the hyperplastic thyroid is particularly liable to release antigens into the circulation with consequent antibody formation or it may be that there is some deeper connection between the aetiology of Graves disease and lymphadenoid goitre

Conclusions

Graves disease has been intensively investigated for a long time but we know little about its causation Presumably this means that the investi- gations have been from the aetiological point of view wrongly aimed It is certainly true to say that the greater part of the work has been focused on the physiological abnormalities resulting from thyroid overactivity Such studies have greatly increased our knowledge of the processes at work in the hyperthyroid patient but while this knowledge is certainly valuable for its own sake it has necessarily little bearing on the funda- mentals of aetiology Attempts to determine whether or not TSH is the

solved this problem The administration of thyroid extract to otherwise normal subjects certainly causes some psychological changes in the same general direction, but in the opinion of many experienced observers never quite reproduces the emotional instability of the natural disease It is difficult to evaluate the trustworthiness of such opinions, and equally so to decide whether the patient with Graves' disease, whose hyperthyroidism has been adequately treated, really reverts to psychological normality Many clinicians think that they do not, but in a careful study Martin[1086] showed that in some apparent examples the symptoms which persisted after a successful thyroidectomy were essentially iatrogenic, for instance, insufficient or inadequate reassurance about the state of the heart had led to the creation of a cardiac neurosis It is perhaps not sufficiently remembered that patients seldom emerge psychologically intact from any severe illness and its attendant treatment The fact that patients are not always emotionally normal when hyperthyroidism has been corrected is not necessarily evidence that they were not emotionally normal before hyperthyroidism began The only satisfactory solution would come from the psychological examination of persons destined to develop Graves' disease in the future if they could be identified Some fragmentary information of this type is supplied by the observations of Fitz[467] who had records of thirty three people examined routinely before the development of Graves disease He was unable to detect any physical or psychological features which would have enabled him to predict the subsequent occurrence of Graves' disease He concluded that Graves disease is a medical misadventure which may befall anyone '

Parry s[1198] second case of what we now call Graves disease was a young woman who was thrown out of a wheelchair coming fast downhill on April 28 1803 From then on she became subject to palpitations, and a fortnight later her thyroid became enlarged This type of anecdote has been repeated countless times in the literature as evidence that Graves disease can be caused or precipitated by emotional upsets of very varied types In fact this type of evidence has little or no value Patients are not uncommonly seen nowadays who undoubtedly have Graves' disease, but who are quite unaware of any symptoms resulting from it they have been diagnosed by alert clinicians either at routine examinations after an incidental minor illness or sometimes from observation at a social meeting Parry s young lady might, for all we can tell, have had Graves disease for many months or years before the bath chair ran away with her To complicate the issue still further patients with Graves disease of which they are not aware, are likely to react with unusual vigour to any emotional upset, and they share with doctors a human tendency to attribute disease to any striking and unpleasant event which happened to precede their symptoms

these observations can be summed up by the hypothesis that for tumour formation in the rat two factors are required (1) some change in the metabolism or genetic constitution of the thyroid cells and (2) stimulation of cell division by TSH

This hypothesis is in some degree applicable to human thyroid cancer The only instance in which an initiating stimulus has been identified comes from the United States where irradiation by X rays in infancy has been followed by thyroid cancer in later childhood in what seems a significantly large number of instances The evidence has been well reviewed by Duffy[407] Controlled studies by Simpson *et al*[1469] and Simpson and Hempelmann[1468] have shown a significant excess of thyroid tumours in children who had been irradiated in infancy for supposed thymic enlarge ment as compared with their non irradiated siblings On the other hand Quimby and Werner[1251] concluded that thyroid cancer rarely occurs in adults who have been treated with X rays for Graves disease On the average the radiation dose received by the adults thyroids was much greater than that received by the children s There is at present no evidence to suggest that irradiation with ^{131}I for Graves disease causes thyroid cancer in the adult although further experience will be required before this possibility can be excluded At first sight these observations suggest that the child s thyroid must be much more radio sensitive than the adult s This may well be true but two other possible explanations need to be considered One is that the thyroid in Graves disease is particularly resistant to the carcinogenic effects of radiation The other suggested by Doniach[391] is that the child s thyroid is under much stronger TSH stimu lation than the adult s He pointed out that the human thyroid enlarges by a factor of about four between the ages of 1 and 12 years From this point of view it could be said that all children have goitres In the adult on the other hand the thyroid cells probably do not divide unless a goitro genic stimulus is present It is generally held that thyroid cancer is com moner in endemic goitre areas although the evidence is by no means clear cut[288] However it is certainly odd that there is not an excessive incidence of cancer among cases of Graves disease where the thyroid cells are undoubtedly being stimulated to divide

There is some evidence to suggest that the promoting action of TSH is required throughout the growth of some tumours In rats some of the tumours appearing after goitrogen treatment could only be transplanted into thyroxine deficient animals but with serial transplantation some of the derived tumours became independent of the hormonal state[1249] Crile[331] has collected instances of human thyroid cancers which appeared to be dependent on thyroxine deficiency for their growth In some cases cancers have apparently been stimulated into rapid growth by surgical or irradiation ablation of the thyroid In a few instances metastases of

immediate cause of the hyperthyroidism of Graves disease belong to much the same category for the solution of this undoubtedly important problem is unlikely to tell us much about the underlying causes of the condition It would seem that some wholly new approach is required To the present writer it appears evident that the most hopeful object of study is the person who is going to develop Graves disease in the future At first sight such a study would appear to require supernatural gifts of divination but this is not necessarily so The genetic evidence is admittedly sketchy, but such as it is it points towards recessive inheritance of a liability to develop Graves disease If we accept this conclusion it follows that an inherited tendency to Graves disease is present in 25% of all affected sibships The siblings of cases of Graves disease therefore constitute a population in which the active disease will certainly appear in a high proportion in the course of time, and should therefore be suitable material for investigation from several different angles Among the possible approaches would be determination of thyroxine disappearance rates, studies on mucopolysaccharide metabolism, serological investigations and personality studies and there are other possibilities An essential feature of this type of investigation would be a long term follow up for active Graves disease may appear at any age For this reason it would be a formidable undertaking to complete Nevertheless the immediate results alone could be expected to provide data of considerable genetic interest

(IV) Thyroid Cancer

The distinction between factors which initiate neoplastic change in a tissue and those which promote the development of an actual tumour in tissues so predisposed, is well illustrated by studies on the thyroid[657] Bielschowsky[168] in relatively short term experiments on rats showed that the combination of a carcinogen (2 acetamidofluorene) and a goitrogen (allylthiourea) produced a large crop of thyroid tumours, whereas neither agent alone did so Later studies by Hall and Bielschowsky[658] showed that if the carcinogen was given early in a course of treatment with a goitrogen tumours developed much more rapidly than in controls treated with the goitrogen alone but after $1\frac{1}{2}$ years treatment the number of tumours was about the same in the two groups Bielschowsky[169] was also able to produce thyroid tumours quite rapidly by subtotal thyroidectomy together with a carcinogen thus demonstrating that any measure causing increased TSH production would promote the growth of tumours

Doniach[388, 389] has demonstrated a similar synergism between irradiation of the thyroid by radioactive iodine and increased TSH secretion induced by treatment with a goitrogen In his experiments the goitrogen alone induced tumours, and so did radioactive iodine by itself, but tumour size and frequency was greatly increased when both agents were included All

APPENDIX

(I) Assay Methods

(a) *Chemical Estimations*

THE chemical determination of iodine in desiccated thyroid and other biological material has been reviewed by Pitt Rivers[1221] and by Charlot and Bezier[280] and readers are referred to these authors for descriptions of the earlier methods The estimation of protein bound iodine in serum has been intensively investigated in recent years and is now carried out by a variety of modifications of Chaney's[275] method The determination entails ashing of organic material with acid (chromic chloric, acid permanganate) or alkali, the reduction of iodine to iodide and its colorimetric estimation resulting from its catalytic action on the rate of reduction of ceric sulphate by arsenious acid Many of these modifications are quoted by Peters and Man[1 13] probably the most commonly used are those of Man and co workers[1213] of Barker[102] and of Zak *et al*[1763] More recent microanalytical procedures for the determination of iodine are found in [865 207 648 969 398 485 810 1411 1606 8 481 1581 189 1113]

(b) *Biological Assay*

Many physiological responses have been used for the quantitative determination of thyroidal potency[691 1289] Some of these methods are of historic interest only and today the following five methods are in most general use the amphibian metamorphosis test the increase in metabolic rate of small laboratory animals the response of the myxoedematous human subject the mouse anoxia test and the goitre prevention test These will be briefly described

(1) *Amphibian metamorphosis test*—Gudernatsch[650 651] first showed that metamorphosis in tadpoles could be accelerated by the administration of thyroid substance the quantitative relationship between dose of thyroid and response was established by Gaddum[554] who showed that the decrease in body length was roughly proportional to the amount of thyroid administered Wokes[1740] showed that the response was linearly related to the logarithm of the dose Shellabarger and Godwin[1456] also use the overall

relatively well differentiated tumours have regressed during treatment with thyroid extract In none of these cases has the causal relationship between treatment and the behaviour of the tumour been established beyond doubt, but in a few the time-relationships are very suggestive From the practical point of view the evidence is certainly strong enough to indicate the potential danger of allowing any patient with thyroid cancer to become hypothyroid for any length of time, but scarcely strong enough to encourage the clinician to rely on thyroid extract as the sole treatment of a thyroid cancer

For more detailed discussion of the aetiology of thyroid cancer the reader is referred to articles by Schlumberger[14 2] Rawson and Benua[1272], Leathem[917] and Doniach[391]

metabolic effect, compounds are also given by intramuscular injection or by the oral route, in single repeated doses In the assay of compounds which are rapidly metabolized the greatest apparent potency will probably be shown after oral administration assuming that the compound is absorbed from the gastro-intestinal tract

(iv) *Mouse anoxia assay*—Duran s[410] observation that the sensitivity of rats to oxygen deficiency was increased when they were made hyperthyroid forms the basis of an assay of thyroidal potency, Smith *et al*[1486] showed that the survival time of mice in closed jars was inversely proportional to the logarithm of the dose of iodinated casein administered this method therefore estimates the rate of oxygen utilization This assay is very simple and has often been used for this reason[247, 16, 4, 569, 45, 1563]

(v) *Goitre prevention assay*—Dempsey and Astwood[365] showed that the hyperplastic changes produced by thiouracil feeding could be reversed by administration of thyroxine and that the decrease in thyroid weight was related to the dose of thyroxine The assay is carried out as follows Rats are given thiouracil or propylthiouracil in the food or drinking water the control animals receive no further treatment groups of the experimental animals are given graded doses of the standard compound (thyroxine) and of the test substance After 10 days the animals are weighed and killed and their thyroid glands are removed The thyroid weights in the various groups (expressed as a percentage of body weight) are plotted against the doses of administered thyroxine and test compound This assay is based on the suppression of thyrotrophin release from the pituitary and potencies obtained by this method will not necessarily enable one to predict activity in metabolic assays although the two types of test do in general give similar results Because of its simplicity the goitre prevention test is now widely used[540, 541, 309, 1624, 7, 644, 1223, 1224, 1132, 299, 882, 1455, 1233, 1442] Mussett and Pitt Rivers[1133] have calculated the relative potencies of thyroxine analogues by the slope ratio method of Finney[484] and the parallel line method and have shown that both calculations lead to similar values

(II) Some Physical Properties of Thyroid Hormones and Related Compounds

Tables IX and X present data of practical usefulness on some physical constants for thyroxine and structurally related compounds Some of these values have been obtained by us the rest are taken from the literature For details of methods and further information the reader is referred to the following papers [1731, 583, 327, 294, 14 1, 14 0, 9, 00, 285, 1221, 1341, 631, 571, 902, 1564, 1346, 42]

decrease in tadpole length for the assay of thyroidal activity in a method in current use today (see [1097]) Other criteria have been employed, such as the appearance of front limb buds[354, 355] and the decrease in width of the tail of the bullfrog, *Rana catesbiana*[242] The most commonly used species are *Rana temporaria* *Rana pipiens* and *Xenopus laevis*

The response of amphibia to thyroid hormones and related compounds is less specific than responses in mammals and for this reason several workers have criticized this method of assay, nevertheless it has a considerable usefulness for the following reasons (1) amphibia seldom respond to a substance which is totally inactive in mammals, so that a qualitative assessment of potency can be obtained from this test, (2) the assay can be performed on large numbers of tadpoles (3) cheaply and (4) rapidly Hence the amphibian metamorphosis test affords a convenient method for the preliminary screening of compounds related to thyroxine

(ii) *Elevation of the metabolic rate in laboratory animals*—Mørch[1101] showed that CO_2 production was raised in mice to which desiccated thyroid had been administered in the same year Gaddum[555] studied the effect of thyroxine on oxygen consumption in rats The closed and open circuit respirometers used in these experiments were described by Mørch[1101] and by Richards and Collison[1300] (see also [1596, 219]), these types of metabolism apparatus are suitable for the smaller laboratory animals (guinea pigs rats and mice) The sensitivity of rats to thyroid and thyroid hormones is considerably enhanced by thyroidectomy[1075] Determinations of oxygen consumption can be made on single animals[98, 722, 88, 1625, 1234] or on groups of animals[1004, 755]

The route of administration of test compounds is of great importance in these and other assays in general intravenous intramuscular intraperitoneal and subcutaneous injections result in a manifestation of greater physiological activity than does oral administration[401, 540, 1100, 4, 8]

Assays of thyroxine like activity by this method generally give values similar to those obtained in humans

(iii) *Response of the basal metabolic rate (BMR) of myxoedematous patients*—From the clinical standpoint the rise in the BMR of myxoedematous subects after the administration of thyroid hormone analogues affords the best method for the determination of their potencies relative to thyroxine [79, 1325, 1404, 1064] The assay of the newer thyroxine analogues (especially triiodothyronine) in humans has been extensively studied both in relation to their overall metabolic effects and their latent periods of action[954, 1275, 65, 951, 300, 1368, 1638, 597, 1733]

In the determination of the latent period of action of a compound it will be administered by the route from which it will be most rapidly distributed to the tissues i e intravenously In studies on the overall

(III) Purification of Thyroglobulin

The method of purification of thyroglobulin now most generally used is that of Derrien, Michel and Roche[374a] This consists of extraction of sliced thyroid tissue with 0 9% sodium chloride solution in the cold, followed by precipitation by addition of ammonium sulphate to a concentration of 42% The precipitate is collected, resuspended in 45% ammonium sulphate and brought back to 37% by addition of water After centrifugation, the insoluble matter is discarded Ammonium sulphate is then added to a concentration of 41% of saturation, the precipitate obtained is suspended in ammonium sulphate at 37% of saturation and centrifuged, the precipitate is again discarded The solution is then dialysed after which thyroglobulin is precipitated with acetone or alcohol These authors have also used a 3 5 M potassium phosphate buffer at pH 6 5 instead of *ammonium sulphate for the precipitation of thyroglobulin* The product so obtained is heterogeneous The validity of the method has been checked by labelling thyroglobulin with ^{131}I[1332a]

Robbins, Wolff and Rall[1323a] have shown that the thyroid contains iodinated proteins that are not salted out by the above procedures using fractional centrifugation and salting out procedures

Simple methods for the partial purification of thyroglobulin have been described by McQuillan *et al*[1061] Kroc, Phillips *et al*[882], Alpers *et al*[39] and Tata *et al*[1577]

Earlier methods for the purification of thyroglobulin can be found in a review by Roche and Michel[1341]

TABLE IX

Polarographic constants ultraviolet absorption and the apparent ionization constant of the phenolic OH of thyroxine and related compounds

Compound	*Polarographic analysis* half wave potentials (volts)	U V absorption maxima wavelength (mμ)	Apparent pK of phenolic OH
L Thyroxine	−1 12 −1 40 −1 70*	231 325	6 45
3 5 3′-Triiodo L-thyronine	—	227 315	8 40
3 5 Diiodo L thyronine	−1 18 −1 36	300	9 30
L-Thyronine	—	295	9 60
3 5 Diiodo L tyrosine	−1 51 −1 70	310	6 50
3 Iodo L tyrosine	—	300	8 70
L Tyrosine	—	290	10 00

* There is much variation in the values obtained by different workers for the first half wave potential of thyroxine[1471, 200, 285, 1544]

TABLE X

Chromatographic mobilities of thyroxine and related compounds

Compound	Relative R_F values		
	Solvent I	Solvent II	Solvent III
L Thyroxine	0 78	0 50	0 42
3 5 3′-Triiodo L thyronine	0 80	0 69	0 50
3 3′ 5′ Triiodo DL thyronine	0 75	0 45	0 48
3 5 Diiodo L thyronine	0 80	0 76	0 50
3′ 5′ Diiodo DL thyronine	0 78	0 50	0 38
3 3′ Diiodo DL thyronine	0 78	0 50	0 42
3 Iodo DL thyronine	0 80	0 62	0 50
3′ Iodo DL thyronine	0 75	0 45	0 47
L Thyronine	0 70	0 58	0 42
3 5 3′ 5′-Tetraiodothyropropionic acid	0 87	0 69	0 60
3 5 3′-Triiodothyropropionic acid	0 88	0 80	0 76
3 5 3′ 5′ Tetraiodothyroacetic acid	0 87	0 68	0 60
3 5 3′ Triiodothyroacetic acid	0 89	0 80	0 74
3 5 Diiodothyroacetic acid	0 90	0 86	0 73
Glucuronide of L-thyroxine	0 10	0 18	0 16
Glucuronide of 3 5 3′ triiodo L thyronine	0 10	0 18	0 20
3 5 Diiodo L tyrosine	0 50	0 08	0 24
3 Iodo L tyrosine	0 35	0 15	0 30

Solvent I = *n* butanol acetic acid water (78 10 12)
Solvent II = *n* butanol dioxane (4 1) saturated with 2N NH_3
Solvent III = collidine water (100 35 5)—Ammonia as vapour phase
Solvent flow ascending Whatman No 1 paper T=18 ±3 C

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